THE

SCIENTIFIC PROCEEDINGS

OF THE

ROYAL DUBLIN SOCIETY.

IMPERIAL M
9 DEC 1942
INSTITUTE

Vol. 22, N.S., Nos. 46–49. MARCH, 1942.

46.—A STRAIN OF THE TUBER BLOTCH VIRUS CAUSING TOP NECROSIS IN POTATO. By PHYLLIS E. M. CLINCH, Albert Agricultural College, Glasnevin. (PLATE 8.)

47.—PHOSPHATE SEPARATION IN QUALITATIVE ANALYSIS. By PROFESSOR J. REILLY and M. O'BRIEN, M.Sc., Chemical Department, University College, Cork.

48.—NOTE ON THE ANALYSIS OF AN IRISH PHOSPHATE ORE. By C. L. SIMPSON, M.Sc., and J. REILLY, M.A., D.Sc., F.I.C.

49.—SALMON OF THE RIVER ERNE. RESULTS OF THE EXAMINATION OF A SMALL COLLECTION OF SCALES AND DATA. By ARTHUR E. J. WENT, Department of Agriculture, Fisheries Branch, Dublin.

[*Authors alone are responsible for all opinions expressed in their Communications.*]

DUBLIN:

PUBLISHED BY THE ROYAL DUBLIN SOCIETY,
BALLSBRIDGE, DUBLIN.

HODGES, FIGGIS, AND CO.,
20 NASSAU STREET, DUBLIN.

WILLIAMS AND NORGATE,
36 GREAT RUSSELL STREET, LONDON, W.C. 1.

1942.

Price Four Shillings.

Royal Dublin Society.

FOUNDED, A.D. 1731. INCORPORATED, A.D. 1750.

The Scientific Meetings of the Society are usually held at 4.15 p.m. on the fourth Tuesday of every month of the Session (November to June).

The attention of authors is directed to the following extracts from the regulations governing the presentation of communications at Scientific Meetings of the Society, and the publication of papers in the Scientific or Economic Proceedings:—

> "An author who desires to present a communication at a Scientific Meeting of the Society shall furnish to the Registrar a fortnight previous to that meeting the title and an abstract (not exceeding 200 words) of his communication, together with an estimate of the time required for presentation.
>
> "An author who desires to have a communication published forthwith as a paper in the Proceedings of the Society shall furnish to the Registrar the full text and necessary illustrations at least a week before the meeting of the Committee of Science at which he desires publication to be considered. Meetings of the Committee of Science are usually held on the second Tuesday of each month from November to June inclusive.
>
> "A paper may be published either in the Scientific Proceedings or the Economic Proceedings of the Royal Dublin Society, as directed by the Committee of Science. The copyright of papers so published shall be the property of the Society.
>
> "Fifty copies of each paper shall be supplied free of cost to the Author or Authors.
>
> "Additional copies may be obtained at Cost Price if applied for by the Author when returning the proofs."

Authors are requested to apply to the Registrar for further information concerning the preparation of papers and illustrations for press.

Authors of biological papers involving extensive numerical observations, are requested to consult the recommendations of the British Association Committee on Biological Measurements, 1927, obtainable from the British Association, Burlington House, W. 1, price 6d. Arrangements have been made whereby original data, too extensive for complete publication, may be deposited at the British Museum (Natural History), or with the Royal Society of Edinburgh, and so be made available for future workers. Authors desirous of depositing data, whether zoological or botanical, in this manner, should communicate with either the Keeper of Zoology, Natural History Museum, S.W. 7, or the General Secretary, The Royal Society of Edinburgh, 22 George Street, Edinburgh, enclosing full bibliographic reference to the publication in which the results are summarised.

No. 46.

A STRAIN OF THE TUBER BLOTCH VIRUS CAUSING TOP NECROSIS IN POTATO.

By PHYLLIS E. M. CLINCH,
Albert Agricultural College, Glasnevin.

(Plate 8.)

[Read June 24. Published separately December 11, 1941.]

Introduction.

The isolation of the tuber blotch virus (syns. virus F, *Solanum virus* 8, *Marmor Aucuba*) was reported from this laboratory in 1935 (17), and a fuller description of its properties and reactions was given in the following year (5). It was concluded that pseudonetnecrosis, as defined by Quanjer, Thung, and Elze (21), is due to the same virus, and that aucuba mosaic (virus G, *Solanum virus* 9) is a closely related form. These observations were confirmed by Dykstra (6), who showed in addition that the newly described Canada streak disease is a strain of aucuba mosaic.

Tuber blotch or virus F was originally isolated by means of the aphis *Myzus persicae* from interveinal mosaic (a complex of viruses F and X), and the previous communication (5) dealt with the virus isolated in this manner. In potato it was characterised by its mild effects on the foliage of twenty-five varieties tested, some of which, however, developed tuber necrosis; typical interveinal mosaic was produced when it was re-combined with virus X.

A second isolation from the X component was made by passage of interveinal mosaic through Arran Crest potato (which reacted with top-necrosis), core grafts of the necrotic Arran Crest tubers to healthy President yielding what appeared to be typical tuber blotch in that variety; when introduced to Irish Chieftain, however, it differed from the virus originally isolated by producing a lethal top-necrosis. Further investigation showed that the units of the stock cultures of interveinal mosaic in President from which the two isolations had been made, displayed a corresponding difference in Irish Chieftain although apparently identical in President.

This threw a new light on an earlier statement by Koch and Johnson (10) that interveinal mosaic received from Ireland in 1932 contained an unknown virus, tentatively named "potato streak," which caused top-necrosis in Bliss Triumph and Green Mountain potatoes. From the knowledge then available here, it was contended (17) that the top-necrosis represented the reaction of the

tuber blotch virus (of whose existence Koch and Johnson were unaware) in these American varieties, the only other virus present in the interveinal mosaic being X, which the American varieties already carried.

The units of interveinal mosaic concerned (including that sent to Professor Johnson) were part of the regular stock cultures of this laboratory and were all descended from a single plant grown in 1928, of which two sister tubers were grown in 1929. By 1932 a descendant of one of these lines contained the top-necrosis element (as shown by Koch and Johnson's tests), and all units derived from it were similarly infected when tested here in 1935. As the parent plant of the clone had proved to be non-necrotic to Irish Chieftain when tested in 1928, the simplest explanation of this difference between its descendants seemed to be that between 1929 and 1932 one of the plants in the stock cultures in the glasshouse had become contaminated with an unknown virus which was carried symptomlessly in President. This implied that the second isolation through Arran Crest was a complex of tuber blotch and the unknown virus (Koch and Johnson's "potato streak"), and steps were accordingly taken to isolate and identify the latter.

In the account of the investigation which follows the supposed complex is referred to for the sake of brevity as "virulent tuber blotch," while true tuber blotch is designated by its synonym, virus F.

Materials.

The source of inoculum for all tests was the stock of President infected with virulent tuber blotch isolated by passage of interveinal mosaic through Arran Crest potato. Infected President plants show little or no reduction in vigour, and foliage symptoms are confined to a slight yellow spotting of the lower leaves (Fig. 1) which, in house plants, is sometimes preceded by an indeterminate mottle.

The tubers show necrosis in the pith and cortex, and in every respect the symptoms are identical with those of true tuber blotch (Virus F) in this variety.

Symptoms in Different Potato Varieties.

The reactions produced when virulent tuber blotch was introduced to a range of potato varieties by graft were of two main types:

1. A rapid and destructive top-necrosis, the stock shoots being completely killed within a few days of the onset of "streaking" in the young leaves (Fig. 2). Tubers, if set, showed severe necrosis which involved the vascular tissues, and destroyed the eyes; the virulent tuber blotch was recovered when such tubers were core-grafted into healthy ones of tolerant varieties.

The following varieties reacted in this manner:—Arran Cairn, Arran Consul, Arran Crest, Arran Peak, Arran Pilot, Arran Signet, Bliss Triumph, Catriona, Di Vernon, Dunbar Cavalier, Dunbar Yeoman, Epicure, Gladstone, Irish Chieftain, King Edward, Majestic, Ulster Monarch, and Up-to-Date.

2. The foliage symptoms were exceedingly mild, and were confined to a slight yellow spotting on the lower leaves (mainly at the distal ends), the vigour of the plants being practically unaffected. In primary infections the yellow spots were in many cases replaced or preceded by reddish-brown glazed areas which dried out and gave a rusty or scorched appearance to the tips and margins of the leaves. In secondary infections, especially under glass, foliage symptoms were sometimes absent altogether. The tubers of several varieties showed necrosis in the cortex and pith, the extent of which varied somewhat even amongst units of the same variety. In the following list of varieties which reacted in this manner, tuber necrosis is indicated by the letter N in brackets :—

Arran Banner, Arran Victory (N), Ballydoon (N), British Queen (N), Champion (N), Doon Early, Doon Star, Dunbar Standard (N), Great Scot (N), Immune Seedling 41956 (N), Kerr's Pink, President (N), Redskin (N), Roode Star (N). (In Arran Victory and Champion the appearance of tuber necrosis was erratic.)

In the varieties included under 2, the effects were identical with those of virus F, which was examined in parallel experiments. Similar mild symptoms were also produced by virus F in all the varieties which were killed by virulent tuber blotch (Group 1), the only exception being Arran Crest. In this variety the young shoots of F-infected plants appear extremely distorted when they come above ground; the leaflets are twisted, and curl downwards, and necrosis of the main veins and petioles prevents their normal expansion. This necrosis is superficial, and dries out after a time; it is confined to the first formed leaves, and later growth is normal. Incidentally, of those varieties in Group 1 which were not included in the previous investigation (5), the following showed tuber necrosis with virus F :—Arran Signet, Dunbar Standard, Gladstone, Redskin, and Ulster Monarch.

Infection of Potato Plants by Sap Inoculation.—Both tolerant and intolerant varieties of potato were inoculated by the rubbing method with virulent tuber blotch. The sap was mixed with powdered carborundum, and 100 per cent. infection was regularly secured in young, actively growing plants. In intolerant varieties, such as Arran Crest, Epicure, and Irish Chieftain, local necrotic lesions appeared after about 9 days, followed later by systemic necrosis. It was sometimes necessary to cut the tops of the plants in order to induce the latter symptom.

Reactions of Virulent Tuber Blotch in Non-Potato Hosts.

Virulent tuber blotch was found to be freely inoculable to the following solanaceous hosts, from all of which it was recovered unchanged on Irish Chieftain potato :—

Capsicum annuum.—Local necrotic lesions are followed by systemic symptoms, which develop acropetally, and consist of vein-clearing, severe mottling, necrosis, and distortion of the leaves, which eventually wither and

drop. Under winter conditions the symptoms are aggravated, and the growing points may be killed.

Solanum nodiflorum.—Conspicuous, purple-fringed circular yellow spots appear on the inoculated leaves; systemic symptoms, which develop acropetally, consist of vein-clearing and conspicuous interveinal chlorosis, followed by rusty reddish discoloration and numerous purple-fringed chlorotic rings. Finally, the leaves turn yellow and drop, but the growing points survive.

Nicotiana glutinosa.—Symptoms appear first in the top of the plant, and consist of veinal chlorosis, followed by interveinal yellow speckling or mottling, and eventual dark green banding of the main veins.

Solanum nigrum.—No local lesions; symptoms appear in the tops of the plants in the form of circular pale green or yellowish areas situated interveinally, and mainly towards the distal ends of the leaves.

Datura Stramonium.—The vigour of the plants is unaffected, but after about 14 days minute raised yellow specks, like intumescences, may be noticed in the interveinal areas of the upper leaves, usually close to the midrib, and towards the proximal end of the leaf; these coalesce and dry out, forming small, irregularly-shaped white patches, reminiscent of thrips injury. The symptoms are confined to those leaves which are in process of development at the time of infection, the plants otherwise appearing perfectly normal. They are of an unusual type, and were originally diagnosed as an effect of virus F by the writer's colleague, Mr. J. B. Loughnane.

Tomato (*var.* Kondine Red); *Petunia* (*var.* Rosy Morn); Tobacco (*vars.* White Burley and Yellow Orinoco).—No symptoms.

The reactions of virulent tuber blotch in the above hosts were identical with those of virus F, as observed in parallel tests. This implied that the top-necrosis element of virulent tuber blotch (assuming the latter to be a complex) was carried symptomlessly in all these hosts.

Physical Properties of Virulent Tuber Blotch.

These tests were repeated twice, and control inoculations with untreated sap were made in each experiment. Except in the dilution tests, the host plants used were *Solanum nodiflorum* and *Datura Stramonium,* which were subsequently grafted on Irish Chieftain potato.

Thermal death point.—5 c.c. samples of crude sap were heated for 10 minutes, then cooled rapidly. A distinct falling-off in the infectivity of the sap was caused by temperatures above 50° C., and no infections were obtained at temperatures above 62° C.

Filterability.—Crude sap, cleared by passing through compressed paper pulp, was filtered through L1, L3, and L5 Pasteur-Chamberland candles. Some infection was obtained from the L1, but not from the L3 or L5 filtrates.

Longevity in vitro.—Crude sap stored in stoppered bottles at 16–18° C. became non-infective after 3–4 days.

Resistance to dilution.—Crude sap, diluted with distilled water, was tested by direct inoculation to Arran Crest potato. A dilution of 1 in 100 considerably reduced the number of local lesions produced, and the limit for infectivity was 1 in 200.

In the foregoing tests the necrotic reaction on Irish Chieftain potato determined the presence of the "top-necrosis element" in the host plants used. Had any separation of virus F from the supposed complex occurred, the Irish Chieftain would have reacted with non-necrotic, yellow mosaic symptoms. Actually no separation took place, and the physical properties of the "top-necrosis element" proved to be similar to those of virus F, as previously determined (5).

COMPARISON OF THE "TOP-NECROSIS ELEMENT" IN VIRULENT TUBER BLOTCH WITH OTHER POTATO VIRUSES CAUSING TOP-NECROSIS.

Koch and Johnson (10) suggested that the virus responsible for the "streak" in their tests of interveinal mosaic might be identical with the top-necrosis virus from Up-to-Date potato, i.e. virus B or *Solanum virus* 4. That the two viruses are quite distinct was shown in the following manner:—

Sap from President infected with virulent tuber blotch was mixed with an equal volume of sap from Up-to-Date infected with B. Samples of the mixture were subjected to filtration, heating, and "ageing," and inoculated after treatment to *Datura Stramonium*; scions from each *Datura* were subsequently grafted on Irish Chieftain and British Queen, the latter being intolerant to virus B.

The results of the tests, which were carried out in duplicate with suitable controls, showed that (*a*) Virus B passed freely through L3 and L5 filter candles, while virulent tuber blotch failed to do so; (*b*) Virus B survived heating at 67° C., while virulent tuber blotch was destroyed at temperatures above 60° C.; (*c*) after 5 days at 20° C. the mixture caused 100 per cent. infection with virus B, but virulent tuber blotch was destroyed. Furthermore, the failure of virus B to "protect" against the "top-necrosis element" was shown by inoculating 12 young Up-to-Date plants with virulent tuber blotch. All of these developed local necrotic lesions followed later by top-necrosis.

Viruses C (*S. virus* 5) and D (*S. virus* 6), described by Bawden (1, 2), produce top-necrosis in many of the varieties similarly affected by virulent tuber blotch. Virus C, however, differs in that it produces top-necrosis in President and Arran Banner, it is not sap-transmissible, and its host range is apparently restricted to potato plants. Virus D has been established as a strain of X, and cannot, therefore, be related to virulent tuber blotch, which differs fundamentally from X in its physical properties, its ability to pass through X-immune seedling 41956, as well as immunologically. (Anti-X rabbit serum failed to induce a precipitin reaction in sap containing virulent tuber blotch.)

Insect Transmission Experiments.

Four experiments were carried out to test whether the top-necrosis element could be transmitted by the aphis *Myzus persicae*. The method was similar in each case, viz.: the insects were colonised on a sprouting tuber of President infected with virulent tuber blotch growing in a dish of moist sand and placed within a larger dish containing the healthy test tubers. The latter, 30 in all, included President (12), Arran Banner (4), Arran Victory (4), Redskin (6), and Epicure (4). The insects were allowed to feed for intervals of 10–18 days, after which the tubers were grown, and, excepting Epicure, all plants were tested by grafting on Irish Chieftain. In no case was any virus transmitted.

In a preliminary experiment to test the effect of using virus A and virulent tuber blotch together, only four experimental tubers were used, viz.: 2 of Irish Chieftain (which carries A) and 2 of President. The insects were allowed to feed for 35 days, and the infestation was heavy. Both Presidents became infected with virus A, and one had virulent tuber blotch in addition. Neither of the Irish Chieftain plants showed foliage symptoms, but a single tuber of one unit had necrotic lesions and the eyes were killed. This suggested infection (possibly of a single stolon) with the "top-necrosis element," but its presence was not proved, as a core-graft from the necrotic tuber to healthy President conveyed only the virus A normally present in Irish Chieftain. In a second experiment in which 3 Irish Chieftain and 9 President tubers were used, the insects were allowed to feed for 14 days. None of the experimental plants showed evidence of virulent tuber blotch in this case.

While confirmation of the ability of *Myzus persicae* to transmit virulent tuber blotch is desirable, it is noteworthy that in the single case where transmission occurred, the virulent tuber blotch was conveyed unchanged, i.e. there was no selective transmission of virus F from the supposed complex. Furthermore, transmission only took place under the conditions already found necessary for transmission of virus F (5), viz., in the presence of virus A and when the aphis infestation was heavy and period of feeding prolonged.

The writer is indebted to Mr. J. B. Loughnane for his co-operation in the foregoing experiments.

Cross Immunity Tests.

The failure to isolate the "top-necrosis element" from virus F in any of the preceding experiments, and the similarity between its fundamental properties and those of virus F, strongly suggested that virulent tuber blotch was not, as originally thought, a complex of two different viruses but was actually a strain of virus F.

It is now generally accepted that one of the strongest criteria of relationship between two viruses is the ability of one to "protect" against subsequent infection with the other. In studies with related strains of other viruses the writer has found that, generally speaking, this protection is absolute only when infection with the second strain is attempted by sap inoculation. If the latter

is introduced by graft, there are obvious signs of resistance to its entry, but systemic invasion usually takes place eventually, a fact also observed by Köhler (13). In order, therefore, to test whether viruses of the F group (5) "protected" against virulent tuber blotch, the following experiments were made:—

(*a*) Ten healthy potato plants (*vars.* Doon Early and Arran Banner) and ten plants of similar age infected with virus F were inoculated with virulent tuber blotch. The inoculations were made when the plants were 4–5 in. high and growing actively, carborundum powder being used to facilitate infection. In due course the originally healthy plants developed reddish, rusty discoloration or mild yellow spotting at the tips of the lower leaves, while the F-infected plants showed slight yellow spotting. Thirty days after inoculation each plant was tested by grafting on Irish Chieftain: all the originally healthy plants produced the top-necrosis reaction characteristic of virulent tuber blotch in this variety, while those infected with F produced only yellow blotches on the leaves, i.e. symptoms of F alone.

(*b*) Five healthy Arran Crest and five infected with virus F were similarly inoculated with virulent tuber blotch when about 6 in. high. As the earlier leaves of F-infected plants are distorted, a second inoculation was made when the latter had developed flat, normal-looking leaves. The control healthy plants developed numerous necrotic lesions (symptomatic of virulent tuber blotch) on all the inoculated leaves, while the F-infected plants failed to show a single lesion.

(*c*) Four vigorous young Early Regent plants infected with aucuba mosaic (virus G) were inoculated with virulent tuber blotch, and 24 days later each was grafted on Arran Pilot. The latter all failed to develop necrosis, showing that none of the Early Regent plants had become infected with the virulent tuber blotch. (Controls could not be used in this case, as Early Regent free from aucuba mosaic is not available.)

(*d*) Nine Doon Early plants (3 infected with F, 3 with virulent tuber blotch, and 3 healthy controls) were inoculated with aucuba mosaic when about 6–8 in. high. At this time the 6 infected plants showed slight yellow spotting on the lower leaves. After 21 days the top leaves of the control plants were covered with a brillant aucuba mosaic mottle, while the tops of the originally infected plants appeared normal and remained so until harvested.

These experiments clearly prove that virus F confers immunity against virulent tuber blotch. The available evidence also indicates that virus G (aucuba mosaic) and virulent tuber blotch are mutually protective.

Conclusions and Discussion.

It has been concluded as a result of the present investigation that the so-called virulent tuber blotch is, in reality, a strain of the normal tuber blotch (virus F) and not a complex of the latter with a contaminating virus of different type. In the first place, its host range and reactions are similar to

those of virus F except in certain potato varieties; in addition the thermal death point, filtration and dilution end points, and longevity *in vitro* of virulent tuber blotch, as such, proved to be identical with those of virus F. Furthermore, absolute "protection" was afforded by virus F against virulent tuber blotch, which is the strongest evidence that the latter does not contain a virus unrelated to F. In addition to these facts, the failure to effect any splitting of virulent tuber blotch by different sap treatments or in numerous inoculation experiments, and the evidence, however slight, derived from the insect transmission experiments, all confirm the belief that only a single virus type is concerned. Whether the normal strain of F co-exists with the virulent strain could not be determined, as the necrotic reaction of the latter in potato constitutes the only known point of difference between the two.

Virulent tuber blotch, therefore, is a new strain to be added to the group of yellow mosaics of potato which already includes tuber blotch (virus F), pseudonetnecrosis and the Monocraat virus (probably identical with F), potato aucuba mosaic (virus G), and Canada streak. By its recognition the "potato streak virus" of Koch and Johnson (10) is identified. Koch and Johnson did not attempt to isolate the virus from the X component of interveinal mosaic, which accounts for the mosaic symptoms observed by them in tobacco.

The identification of virulent tuber blotch as a strain of virus F raises the interesting question of virus mutation. It has been pointed out that the different units of President potato from which F and virulent tuber blotch were derived belonged to one clone, all the members of which were propagated by tuber under glass. The parent plant of the clone contained virus F, and was non-necrotic to Irish Chieftain, but from one of two plants grown in 1929, a direct descendant contained the top-necrosis element, i.e. virulent tuber blotch, in 1932. Accepting the evidence that the latter is indeed a strain of virus F, and that the two strains confer immunity one against the other, it follows that virulent tuber blotch could not have been introduced as a contaminant from outside sources owing to the prior presence of F; nor could it have appeared as the result of natural selection from a pre-existing mixture of virus strains in the stock. Consequently, it may be assumed that a spontaneous change of virus F to virulent tuber blotch took place naturally within the tissues of a potato plant, but whether the change occurred in the aerial portion during the growing season or in a tuber during the dormant period is quite unknown. However it arose, the variant has remained stable in potato for several years, and has not been changed by passage through other solanaceous hosts. It differs from the original virus F only in one property, viz., the ability to produce top-necrosis in certain potato varieties.

In the case of tobacco mosaic and cucumber mosaic viruses, many new strains have been isolated by sub-inoculation from yellow spots appearing on the leaves of infected plants. The work of McKinney (19), Jensen (8, 9), Kunkel (16), and Price (20) proved beyond reasonable doubt that the viruses arising in such spots are not due to contaminants in the inoculum, but are formed spontaneously in some unknown manner from the original infecting

virus; they represent permanent departures from type, and presumably may be regarded as mutants. In addition to these well-established cases of strain variation in localized areas of infected leaves, Jensen (9) observed that, following inoculation of groups of plants with a pure strain of yellow tobacco mosaic virus, an occasional plant showed systemic infection with a different strain. Hoggan (7) had a similar experience in the course of aphis transmission experiments with cucumber mosaic virus, and both investigators believed that the new viruses arose spontaneously from the original infecting virus. Salaman (22) effected conversion of potato virus X from severe to mild forms by sap inoculation of tobacco and continued passage through the same host, as well as by passage through horse beans and other non-solanaceous hosts, and he regarded these conversions as true mutations.

Böhme (3, 4) and Köhler (11, 12, 14) isolated several different types of virus X from field potatoes, and they were of opinion that the X virus undergoes permanent modifications within the tissues of the living plant. Observing later that certain X virus strains readily gave rise to different variants in tobacco, Köhler (15) visualized these changes as due to graduated associations and dissociations of the molecules of the virus protein. Salaman (22) expressed the view that the different clinical types of virus X isolated by Böhme and Köhler from potatoes were, in reality, mixtures of strains, and stated that the X virus content of most naturally infected potato plants is rarely confined to a single strain. In spite of the fact that related strains are mutually protective, Salaman hesitated to invoke mutation within the tissues as the explanation of such mixed infections, believing it more probable that field plants infected with mixed strains received such mixtures either simultaneously in a single infection, or in two or more individual infections at short intervals. The existence of a vector of virus X appears to be implied by Salaman, but so far none has been discovered, and the virus is only known to be transmitted by contact between healthy and diseased plants (18). Under the circumstances the initial occurrence of mixed strains of virus X would be more readily explained, in the writer's opinion, on the grounds of spontaneous variation of virus within the tissues of the living plant.

In the present instance, the virulent tuber blotch strain of F arose independently of any artificial treatment, and its production must necessarily have been determined by factors operating *in vivo*. Although the systemic occurrence of a new strain in this manner constitutes an unusual phenomenon in our experience of virus-infected potato plants propagated under glass, the mixed strain infections which have been observed in field plants may be further instances of the same phenomenon.

SUMMARY.

A "top-necrosis element" was found to be present in certain units of the stock cultures in President potato of interveinal mosaic, previously shown to

be a mixture of viruses F (tuber blotch) and X. As the parent plant of the clone and other units descended from it were free from this element, the latter was assumed to be a contaminant.

Removal of the X component from interveinal mosaic showing the top-necrosis reaction yielded a supposed complex of the "top-necrosis element" and virus F, referred to as virulent tuber blotch.

The reaction of virulent tuber blotch in 32 varieties of potato was determined: 18 were killed by top-necrosis, and 14 showed a mild reaction identical with that of virus F.

The reactions in 8 other solanaceous hosts, as well as the physical properties of virulent tuber blotch, were found to be similar to those of virus F.

Evidence that the "top-necrosis element" is not related to the potato viruses B, C, or D, which also cause top-necrosis, is presented.

The aphis *M. persicae* failed to transmit any virus from President infected with virulent tuber blotch alone. In the presence of virus A (a condition necessary for the transmission of F), virulent tuber blotch, as such, was transmitted in a single case.

Potato plants infected with virus F were immune to infection with virulent tuber blotch. Virus G (aucuba mosaic) and virulent tuber blotch were also mutually protective.

It was concluded that virulent tuber blotch is a strain of virus F (*Solanum virus* 8, *Marmor Aucuba*), which arose spontaneously from the latter within the tissues of a potato plant. By its recognition, Koch and Johnson's "potato streak" virus is identified.

Cases of virus strain conversion in the literature are discussed.

EXPLANATION OF PLATE.

Fig. 1.—Potato *var.* President infected with virulent tuber blotch, showing slight yellow mottle at tips of lower leaves.

Fig. 2.—Potato *var.* Irish Chieftain infected with virulent tuber blotch, showing top-necrosis.

REFERENCES.

(1) Bawden, F. C.—Proc. Roy. Soc., B, **116**: 375–395, 1934.
(2) ————————Ann. App. Biol., **23**: 487–497, 1936.
(3) Böhme, R. W.—Phytopath. Z., **6**: 453–542, 1933.
(4) ————————Arb. Biol. Reichsanst. für Land. und Forstwitsch., **21**: 1–58, 1934.
(5) Clinch, P. E. M., Loughnane, J. B., and Murphy, P. A.—Sci. Proc. R.D.S., **21** (N.S.): 431–448, 1936.
(6) Dykstra, T. P.—Phytopath., **29**: 917–933, 1939.
(7) Hoggan, I. A.—Ann. App. Biol., **22**: 27–36, 1935.
(8) Jensen, J. H.—Phytopath., **23**: 964–974, 1933.
(9) ————————*Ibid.*, **26**: 266–277, 1936.

(10) KOCH, K., and JOHNSON, J.—Ann. App. Biol., **22**: 37–54, 1935.
(11) KÖHLER, E.—Phytopath. Z., **7**: 1–30, 1934.
(12) ————————Naturuwissenschaften, **49**: 828–830, 1935.
(13) ————————Angew. Bot., **17**: 61–74, 1935.
(14) ————————Phytopath. Z., **10**: 31–41, 1937.
(15) ————————*Ibid.*, **10**: 467–479, 1937.
(16) KUNKEL, L. O.—Phytopath., **24**: 437–466, 1934.
(17) LOUGHNANE, J. B., and CLINCH, P.—Nature, **135**: p. 833, 1935.
(18) ———————— and MURPHY, P. A.—Sci. Proc., R.D.S., **22** (N.S.): 1–15, 1938.
(19) MCKINNEY, H. H.—J. Agr. Res., **51**: 951–981, 1935.
(20) PRICE, W. C.—Phytopath., **24**: 743–761, 1934.
(21) QUANJER, H. M., THUNG, T. H., and ELZE, D. L.—Meded. Landbouwhoogesch., **33**: 1–10, 1929.
(22) SALAMAN, R. N.—Phil. Trans. Roy. Soc., B, **229**: 137–217, 1938.

FIG. 1.

FIG. 2. [*Photos by G. H. McLean.*

No. 47.

PHOSPHATE SEPARATION IN QUALITATIVE ANALYSIS.

By PROFESSOR J. REILLY and M. O'BRIEN, M.Sc.,
Chemical Department, University College, Cork.

[Read November 25, 1941. Published separately February 11, 1942.]

In the schemes of qualitative analysis most widely used it is necessary to remove phosphate ions, if present, before the iron-aluminium group of metals can be separated from the alkaline earth group. Of the various methods proposed for the removal of the phosphate ion, only two are widely used, namely the tin method and the ferric chloride method. These are to some extent unsatisfactory. They all have certain disadvantages—such as incompleteness of removal, difficulty of technique, loss of other ions, etc. For example, the tin method gives incomplete removal, except when a large amount of tin is used. On the other hand, the use of large amounts of tin causes an appreciable loss of other ions. Curtman (1) found that if more than 2 g. of tin were used there was almost complete loss of iron and barium. The ferric chloride method is generally recognised as a long operation, involving large volumes and giving incomplete removal unless carried out with great accuracy. In this method also there is loss of other ions (1).

The zirconium oxychloride method, which was devised by Curtman (1), is more satisfactory than either of the above. Removal by this method depends on the fact that zirconium forms a phosphate which is insoluble in strongly acid solution, and hence the phosphate ion can be removed from solution by the addition of a soluble zirconium salt. The reagent used in removal by this method is zirconium oxychloride ($ZrOCl_2$).

To the filtrate from the copper and tin groups add 2 g. of ammonium chloride and 10 ml. of zirconyl chloride solution (50 mg. of Zr . . . . per ml.) drop by drop, stirring vigorously. Add 0·2 g. of asbestos or one Fisher filtration accelerator. Render alkaline with ammonium hydroxide, and boil for 2 minutes. Neutralize with dilute hydrochloric acid, add 10 ml. of 3N hydrochloric acid in excess, and boil for 3 minutes. Filter hot on a fluted filter.

Addition of the zirconium oxychloride solution gives a white insoluble precipitate of zirconyl phosphate. The reagent is added dropwise, as rapid addition causes incomplete removal, and the precipitate formed is usually colloidal (2). The solution is agitated during addition to prevent colloidal formation. Excess zirconium is co-precipitated with the phosphate as zirconium hydroxide by adding ammonium hydroxide and boiling. The metals of the iron-aluminium group are also precipitated at this stage. Curtman, however, claims (3) that on boiling with hydrochloric acid in the next step, the

zirconium hydroxide does not redissolve, and in this way excess zirconium can be removed. Pittmann refutes this claim (2), and asserts that it will completely dissolve under the conditions of the experiment.

The following (2) are the main disadvantages of the Curtman procedure:—

1. Notwithstanding dropwise addition of the reagent, agitation of the solution, and the presence of ammonium chloride, the precipitate is largely colloidal. This introduces difficulty in filtering.
2. There is a large loss of other ions.
3. Usually there is a large excess of reagent, which interferes in later tests.
4. The method of removal of excess reagent is unsatisfactory. Most of it comes down with the iron-aluminium group, and interferes in the detection of these metals.

In an effort to eliminate these difficulties, Pittmann proposed the following modified procedures:—

The filtrate from the acid hydrogen sulphide precipitation (copper-tin group) is boiled until all hydrogen sulphide has been expelled, and the volume of the resulting solution is adjusted to approximately 100 c.c. The cooled solution is neutralized with ammonium hydroxide, and then made acid with 5 c.c. of 6N nitric acid. To this acid solution 35 c.c. of 0·015 M zirconyl chloride solution (for every 40 mg. of phosphate) are added, a few drops at a time, with vigorous stirring during the entire addition. The stirring is continued for a few seconds, and then the precipitate is allowed to settle for 1 minute. The mixture is filtered on a very porous filter paper, such as Delta 366, without suction. As much of the supernatant liquid as possible is poured through the paper before the main body of the precipitate is added. The precipitate is washed several times with small amounts of cold water, or, even better, with a 5 per cent. solution of ammonium nitrate, and the washings are collected in the same beaker with the main portion of the filtrate. The precipitate is rejected. The solution now contains less than 1 mg. of phosphate, and in most cases the small amount remaining can be disregarded. However, if it is desired to remove the last traces, 10 c.c. of 0·05 M zirconyl chloride solution are added to the filtrate, and the mixture is heated to just below boiling and held at that temperature for about 2 minutes. The solution is allowed to stand for 5 minutes and filtered.

The aim of the present work is to investigate the efficiency of the Pittmann procedure. It will be shown that it has disadvantages. In an effort to overcome these the factors responsible for them have been studied and eliminated, and a modified procedure for the removal of phosphate by means of zirconium oxychloride has been proposed.

Experimental.

Several phosphate removals were made by the Pittmann procedure, and in each case the following points were noted:—nature of precipitate, time of filtration, nature of filtrate, amount of excess reagent, volumes involved. The percentage removal was also determined by estimating the phosphate in the filtrate after removal. This was done by the hydroquinone colormetric method (4), using a Klett colorimeter. The efficiency of this method of phosphate estimation was first tested with standard phosphate solutions.

The result of many experiments showed that the Pittmann procedure has the following disadvantages:—

1. There is incomplete removal, only 94 per cent. (approx.) of the phosphate being removed.

2. Notwithstanding careful addition of the reagent and vigorous stirring throughout, the phosphate precipitate is, as in the Curtman procedure, largely colloidal. Pittmann asserts that it settles quickly, and filters easily. The contrary has been found to be the case in the present investigation. In no case did the precipitate completely settle, even after allowing it to stand for 2 hours.

3. On account of the weak solution of zirconyl chloride employed (0·48 per cent.), and due to the fact that the filtrate from group 2 must be adjusted to 100 c.c. before removal, large volumes are involved, which necessitate tedious evaporation after removal.

4. Due to colloidal formation, and the large volumes involved, much difficulty is encountered in filtering. Moreover, the filtrate is cloudy.

5. Precipitation of excess zirconium with ammonium hydroxide showed that considerable excess of reagent is used. Apart from the consideration of cost, the use of excess zirconyl chloride is a disadvantage, as it interferes in the detection of the iron-aluminium group.

6. The Pittmann procedure presumes that the amount of interfering phosphate present is approximately known, which in most analyses is not the case.

For a satisfactory method of removal of phosphate by means of zirconium oxychloride, the following conditions must be fulfilled:—(*a*) complete removal, (*b*) elimination of colloidal precipitation, (*c*) small volumes, (*d*) absence of large excess of reagent, (*e*) no serious loss of other ions. The following investigation was undertaken in order to obtain these conditions.

It has already been stated that large volumes are involved in removal by the Pittmann procedure. The following example will illustrate this:—

In carrying out his instruction as to the volume of reagent to be added (35 c.c. for every 40 mg. of phosphate), a solution containing 0·14 g. of phosphate required 122·5 c.c. of the reagent. The volume of the filtrate after removal of the phosphate was approximately 240 c.c.

The use of large volumes is undesirable for the following reasons:—

1. As the reagent must be added dropwise, and with vigorous stirring, addition of large volumes is a slow and tedious operation. It was found, as a result of several trial runs, that addition of the reagent in the removal of 0·14 g. of phosphate takes *at least 30 minutes.*

2. Owing to the fact that the phosphate precipitate is largely colloidal, and does not settle, the use of large volumes increases the difficulty of filtration.

Filtration after removal of 0·14 g. of phosphate took approximately 1½ hours in every case.

3. The use of large volumes necessitates tedious evaporation after removal. For example, after removal of 0·14 g. of phosphate, the filtrate must be reduced from approximately 240 c.c. to 10 c.c. (approx.) in order to obtain a volume which can be conveniently handled in the analysis for the remaining metals.

(A) In an effort to accelerate the removal and obviate evaporation, the following procedure was adopted:—

(*a*) Instead of adjusting the volume of the filtrate from Group 2 to 100 c.c. a volume of approximately 20 c.c. was used.

(*b*) A 10 per cent. solution of zirconium oxychloride was used instead of a 0·48 per cent. solution (0·015 M), as used in the Pittmann procedure. 10 c.c. of the reagent was added.

The following results show that the modified procedure was a distinct improvement:—

	Time of addn. of reagent.	Time of filtration.	Vol. of Fil. after removal.	Time of removal.
Pittman Procedure ...	30 mins.	85 mins.	240 c.c.	2 hrs.
Modified Procedure ...	2½ mins.	20 mins.	40 c.c.	27½ mins.

(B) A serious disadvantage of the Pittmann procedure is the fact that the phosphate precipitate is largely colloidal. This makes filtration very difficult, and gives a cloudy filtrate.

In order to ascertain the stage at which colloidal precipitation began, 0·14 g. of phosphate (as potassium dihydrogen phosphate) was removed by the Pittmann procedure, but the precipitate was allowed to stand for a few minutes after addition of every 10 c.c. of the 0·015 M zirconyl chloride solution, and examined. It was observed that after addition of 80 c.c. of the reagent, the precipitate was granular, and settled quickly. On adding a further 10 c.c., however, colloidal formation was distinctly indicated. The mixture now became milky; the precipitate lost its granular appearance, and did not settle after allowing it to stand for 30 minutes.

The same weight of phosphate (0·14 g.) was again precipitated, but this time only 80 c.c. of the reagent was added. The mixture was now filtered, time of filtration noted, nature of filtrate observed, and the filtrate was boiled with ammonium hydroxide, to precipitate excess zirconium, if present. The results

are given in the following table, together with the corresponding results after addition of the volume of reagent prescribed by Pittmann (122·5 c.c.).—

	80 c.c. of 0·015 M $ZrOCl_2$ solution added.	122·5 c.c. of 0·015 M $ZrOCl_2$ solution added (Pittmann procedure).
Nature of precip. ...	granular	largely colloidal
Time of filtration ...	25 minutes	85 minutes
Nature of filtrate ...	clear	cloudy
Excess reagent ...	very slight excess	large excess.

The fact that the filtrate after removal of the phosphate by addition of 80 c.c. of the reagent gave a very slight precipitate of zirconium hydroxide on boiling with ammonium hydroxide, showed that 80 c.c. of the 0·015 M zirconyl chloride solution was just sufficient to completely precipitate the phosphate. It also showed that a considerable excess of reagent is used in the Pittmann procedure, and suggested that *colloidal formation might possibly be due to this excess.*

(C) The stage at which colloidal formation begins during removal by the modified procedure (i.e. adjusting the volume of the filtrate from Group 2 to 20 c.c., and adding a 10 per cent. solution of zirconyl chloride) was now investigated. 0·14 g. of phosphate was again used, and the 10 per cent. solution of the reagent was added dropwise and with vigorous stirring. After adding 3·5 c.c. of the reagent, the mixture was filtered. A further 0·2 c.c. was now added to the filtrate, and the mixture was again filtered. This filtrate was again treated with 0·2 c.c. of the reagent, and filtered, and the operation was repeated until precipitation was complete. In each case the nature of precipitate and filtrate was observed.

The following results were obtained:—

(*a*) Phosphate soln. + 3·5 c.c. reagent—granular ppt., clear filtrate.
(*b*) Filtrate from (*a*) + 0·2 c.c. reagent— „ „ „ „
(*c*) „ „ (*b*) + 0·2 c.c. „ — „ „ „ „
(*d*) „ „ (*c*) + 0·2 c.c. „ —no pption. or cloudiness.

Addition of a further 3 c.c. of reagent to the filtrate from (*c*) gave no precipitation or cloudiness.

3·7–3·9 c.c. of a 10 per cent. solution of zirconyl chloride *just* precipitates 0·14 g. of phosphate. Note that no colloidal precipitation was observed in this experiment. Note also that, although a considerable excess of reagent was added, at no time was there excess of reagent with the phosphate precipitate. This strengthens the possibility that colloidal formation is due to excess reagent.

(D) 0·14 g. of phosphate (in a solution of 20 c.c.) was now *just* precipitated by addition of 3·9 c.c. of the 10 per cent. zirconyl chloride solution, and the

mixture was filtered after allowing it to stand for a few minutes. This resulted in

(*a*) A heavy granular precipitate which settled quickly, giving a clear supernatant liquid.
(*b*) Time of filtration—7 minutes.
(*c*) Clear filtrate.

In order to see the effect of excess reagent on the phosphate precipitate, the same weight of phosphate (0·14 g.) was again *just* precipitated by addition of 3·9 c.c. of the 10 per cent. solution of zirconyl chloride. A heavy precipitate was again obtained, which settled quickly, to give a clear supernatant liquid. 3 c.c. (excess) of reagent was now added dropwise and with vigorous stirring. Distinct colloidal formation was observed; the precipitate lost its granular nature, and a milky "solution" was obtained. The precipitate did not now completely settle, even after allowing it to stand for several hours. Filtration was very difficult, and a cloudy filtrate was obtained.

The results of Experiments **B**, **C**, and **D** prove conclusively that *colloidal precipitation is due to excess reagent.*

Pittmann states (2) that if precipitation is made from a hot solution, or if precipitation is made from a cold solution, and the mixture then heated to boiling, the tendency is very markedly towards the formation of a colloidal suspension. This was tested for the case where large excess of the reagent is not added.

(E) 0·14 g. of phosphate was just precipitated from a hot solution by addition of 3·9 c.c. of a 10 per cent. solution of zirconyl chloride. As a result—

(1) No colloidal formation was detected. A heavy precipitate was formed, which settled in a few minutes, to give a clear supernatant liquid.

(2) The mixture filtered in 5 minutes, and a clear filtrate was obtained.

(F) 0·14 g. of phosphate was then just precipitated from a *cold* solution by addition of 3·9 c.c. of the 10 per cent. solution of zirconyl chloride. The mixture was heated to boiling, and then allowed to stand for a few minutes before filtering.

(1) No colloidal formation was detected. The phosphate precipitate settled quickly, and the supernatant liquid was clear.

(2) The mixture filtered in 7 minutes, giving a clear filtrate.

(G) After removal of 0·14 g. of phosphate (in 20 c.c. of solution, as in **C**, **D**, **E**, and **F**) by addition of 3·9 c.c. of the 10 per cent. solution of reagent, the mixture was boiled for half a minute, and filtered hot. The time of filtration was 2½ minutes. Clear filtrate.

The results of experiments **E** and **F** show that, contrary to Pittmann's assertion, neither precipitation from a hot solution, nor heating of the mixture to boiling after precipitation in the cold, causes colloidal formation, *provided*

excess of reagent is not added. It is seen from experiment **G** that boiling the mixture after precipitation and filtering hot considerably accelerates filtration.

Pittmann states that if the solution is not agitated during precipitation, the tendency is toward formation of a colloid (2). This was now tested for the case where excess of reagent is not added.

(H) 0·14 g. of phosphate was just precipitated by addition of 3·9 c.c. of the 10 per cent. zirconyl chloride solution. The reagent was added dropwise, but *without stirring.* A heavy precipitate was obtained, which settled *immediately.* On boiling the mixture and filtering hot a clear filtrate was obtained. Time of filtration—3 minutes.

In the foregoing investigation, many factors which were supposed to influence colloidal formation have been studied. It has been proved to be due solely to addition of excess reagent. In previous investigations this cause seems to have been overlooked. It is seen that, provided excess of zirconyl chloride is not added, neither precipitation from a hot solution, nor boiling after precipitation in the cold, will cause difficulty due to colloidal formation. On the contrary, boiling the mixture after precipitation, and filtering hot, enables the filtration to be carried out in a few minutes (Expt. **G**). It is also seen that agitation of the solution is unnecessary when excess of reagent is not added (Expt. **H**).

Is the phosphate completely removed by the Pittmann procedure? This important question was next studied. The percentage removal was determined as follows:

(K) A known weight of phosphate (0·14 g.) was dissolved in water, and, after removal of the phosphate in strict accordance with Pittmann's instructions, the bulk of the filtrate was reduced on a water-bath, and the volume then accurately adjusted to 100 c.c. The amount of phosphate in the filtrate was then estimated by the hydroquinone colorimetric method (4), using a Klett colorimeter. The efficiency of this method of phosphate estimation was first tested with standard phosphate solutions, and it was found to be sufficiently accurate for the purpose.

The results showed that approximately *6 per cent. of the phosphate is unremoved.* The immediate problem, therefore, was to discover the cause of incompleteness of removal.

(L) In the latter experiment the phosphate was removed by addition of 122·5 c.c. of an 0·015 M zirconyl chloride solution in accordance with Pittmann's instruction (35 c.c. for every 40 mg. of phosphate). Removals of the same weight of phosphate (0·14 g.) were now carried out by the addition of (*a*) 80 c.c., (*b*) 100 c.c., (*c*) 155 c.c., (*d*) 175 c.c., (*e*) 200 c.c., respectively, of the 0·015 M zirconyl chloride solution, and the percentage phosphate unremoved was determined in each case by the method already described. The following important results were obtained:—

Volume of 0·015 M $ZrOCl_2$ Solution added.	Percentage phosphate unremoved.
80 c.c.	0·2
100 c.c.	2·4
122·5 c.c.	5·9
155 c.c.	7·1
175 c.c.	7·4
200 c.c.	7·7

It is seen that (*a*) 80 c.c. of the reagent causes practically complete removal, (*b*) the percentage phosphate unremoved increases with the volume of reagent added in excess of 80 c.c.

It has already been found (Expt. **B**) that 80 c.c. of an 0·015 M zirconyl chloride solution just precipitates 0·14 g. of phosphate. It is clear, therefore, that incomplete removal is caused by addition of excess reagent. The fact that the percentage of phosphate unremoved increases with the amount of excess reagent added suggests that this excess of zirconyl chloride forms some soluble complex with the phosphate precipitate. On plotting a graph showing the relation between the volume of reagent added, and the percentage phosphate unremoved, a smooth curve was obtained. A study of the curve showed that the percentage phosphate unremoved approaches a limit (roughly 8 per cent.). This would be explained by the assumption that excess zirconium oxychloride forms a partly soluble complex with the phosphate precipitate. The point at which the maximum amount of phosphate is in solution is reached when the amount of excess zirconyl chloride added is sufficient to convert *all* the insoluble phosphate precipitate into the partly soluble complex. It follows that any further addition of reagent will have no effect on the amount of phosphate in solution.

(M) The completeness of removal by the modified procedure was now examined. 0·14 g. of phosphate (as potassium dihydrogen phosphate) was dissolved in water, and the volume of the solution adjusted to approximately 20 c.c. The phosphate was now just precipitated, by slow dropwise addition of 3·9 c.c. of a 10 per cent. solution of zirconyl chloride, with vigorous stirring throughout the operation. The mixture was then heated to boiling, and filtered hot. The precipitate was washed thoroughly, and the washings collected with the main filtrate. The volume of the filtrate was now accurately adjusted to 100 c.c., and the amount of phosphate present estimated by the hydroquinone colorimetric method. No colour could be detected in the colorimeter. As the method is claimed to be sufficiently sensitive for the estimation of 0·01 mg. of phosphate, the result showed that more than 99·9 per cent. of the phosphate had been removed.

Conclusion. Excess reagent causes incomplete removal. The phosphate is completely eliminated only when excess reagent is not added.

It has been shown that two of the most serious disadvantages of the zirconium oxychloride method of phosphate removal, as carried out by Curtman and Pittmann, namely, incomplete removal and colloidal formation, are due to excess reagent. As a result of the foregoing investigation, the following conditions have been found to give complete removal of the phosphate in an easily filterable condition, and to obviate tedious evaporation after removal:—

(1) Adjust the volume of the filtrate from Group 2 to approximately 20 c.c.

(2) Add a 10 per cent. solution of zirconyl chloride, a few drops at a time, until the phosphate is *just* completely precipitated.

As the success of the method, from the standpoint of efficiency and ease of execution, depends on the condition that excess of reagent is not added, the problem now was to devise a means by which the point of complete precipitation could be ascertained.

(N) The various adsorption indicators—fluorescein, eosin, tartrazine, etc.—were used without success. None of them gave a colour change on complete removal of the phosphate.

(P) The possibility of being able to detect the point at which addition of one drop of the reagent gave no precipitate, by means of a coloured background, was then studied. The filtrate from Group 2 was highly coloured by different colouring agents (e.g., methylene blue, magenta, etc.), and the reagent was added drop by drop. It was found that when the solution was coloured deep blue or brown, the point of complete precipitation could be satisfactorily determined. Over the dark-coloured background of the solution, the white colour of the precipitate formed on addition of each drop of reagent was plainly visible momentarily, but was destroyed on stirring. The procedure, therefore, was to produce a deep blue or brown coloration of the solution by addition of some suitable colouring matter. Then add the reagent, a few drops at a time, stirring after each addition, until addition of a few drops causes no temporary white coloration on the surface of the solution. This method was, however, deemed unsatisfactory, as it necessitates the introduction of foreign matter into the solution, which is undesirable. Moreover, the heavy colour of the solution must be destroyed, as it interferes in later stages of the analysis.

(Q) The following procedure for determining the end point was then tried, and found to be very satisfactory:—

The filtrate from Group 2 was transferred to a boiling tube (small type), and the volume adjusted so that it filled approximately half the tube. The 10 per cent. zirconyl chloride solution was then added from a burette, a few drops at a time, *without stirring*. The precipitate formed on addition of each few drops settled immediately. After each addition the precipitate formed was allowed to settle before further addition. The end-point was reached

when addition of a few drops of the reagent caused no precipitation. The aim in using the boiling tube was to get a large depth of solution with a volume of 20 c.c.–30 c.c., so that an appreciable column of clear supernatant liquid would be obtained during precipitation. One difficulty encountered was that the precipitate, being very flocculent, sometimes completely fills the solution before the end-point is reached, giving no supernatant solution. This was overcome by the following procedure:—If the precipitate becomes too bulky before the end-point has been reached, or if it does not completely settle, boil the mixture for one minute and allow to stand. The precipitate will now settle in a few minutes in much smaller bulk, giving an appreciable column of clear supernatant solution.

As 4 or 5 drops (approx. 0·5 c.c.) of excess reagent might possibly be added by this method, the extent to which this small excess affects (*a*) completeness of removal, (*b*) colloidal formation, was examined.

(R) After adjusting the volume of a solution containing 0·14 g. of phosphate to half-fill a small boiling tube, *4·6 c.c.* of a 10 per cent. solution of zirconyl chloride (approx. *0·7 c.c. in excess*) was added dropwise, without stirring. The mixture was then heated to boiling, and filtered hot. The amount of phosphate in the filtrate was estimated by the hydroquinone colorometric method.

(*a*) Time of filtration—5 minutes.
(*b*) Clear filtrate.
(*c*) 99·6 per cent. of the phosphate was removed.

(RI) The same weight of phosphate was removed by the same method, but this time 5 c.c. of the reagent (approx. 1·1 c.c. in excess) was added.

(*a*) Time of filtration—5 minutes.
(*b*) Clear filtrate.
(*c*) 99·3 per cent. of the phosphate was removed.

The results of experiments **R** and **RI** show that *small excess of reagent does not affect the efficiency of this method from a qualitative standpoint.*

Proposed Procedure.

On the results of the foregoing investigation, the following procedure is proposed for the removal of all the phosphate present by means of zirconium oxychloride. The technique is simple; small volumes are involved, which obviate tedious evaporation after removal; there is complete removal, and a very slight excess of zirconium, which minimises its interference in later stages of the analysis. Moreover, all filtration difficulties due to colloidal formation are overcome:—

Transfer the solution to a boiling tube (small size), and adjust the volume so that it approximately half-fills the tube. Add 2 g. of ammonium chloride,

add a 10 per cent. solution of zirconyl chloride, a few drops at a time, without stirring or shaking. Allow the precipitate formed on addition of each few drops of the zirconyl chloride solution to settle before further addition. Continue adding until addition of a few drops to the clear supernatant solution causes no precipitation. Heat the mixture to boiling and filter hot. Treat the filtrate from this precipitation by the same procedure that would have been followed had phosphate been absent.

Note.—If the precipitate becomes too bulky before the end-point is reached, or does not completely settle, boil the mixture for one minute, and then allow to stand. It will now settle in much smaller bulk. (If the mixture after boiling is milky, and does not settle after 10 minutes, excess zirconyl chloride has definitely been added. Heat the mixture to boiling and filter hot.)

Excess Reagent.—Excess zirconium is precipitated with the iron-aluminium group, and will follow the iron. The small excess can be ignored, as it will cause no difficulty in the tests for that element by the usual ferrocyanide or thiocyanate methods. If, however, it is desired to isolate the excess zirconium, the following procedure can be adopted:—

To the filtrate obtained after removal of the phosphate, add ammonium hydroxide till alkaline. This precipitates iron, aluminium, and chromium, if present, and excess zirconium, as hydroxides. Boil to complete precipitation. Filter and wash precipitate. The filtrate contains the metals of Groups 4–5. Reserve.

Ppt. Fe $(OH)_3$—red; Cr $(OH)_3$—green; Al $(OH)_3$—white; $Zr(OH)_4$—white. Wash into a boiling tube with cold water. Add carefully to the cold mixture Na_2O_2 in separate small quantities until the mixture is strongly alkaline. Heat *gently* till reaction ceases (no further evolution of gas). Filter and wash residue remaining on the paper.

Residue:—$Fe(OH)_3$. Dissolve in a little warm HCl and test solution for iron.

Filtrate $NaAlO_2$, Na_2CrO_4, Na_2ZrO_3. Boil for 2 minutes, filter, and wash residue.

Residue:—$Zr(OH)_4$.

Filtrate $NaAlO_2$, Na_2CrO_4. Separate and detect by the usual methods.

Loss of Ions.—In both the tin and ferric chloride methods, there is a considerable loss of ions (1). The Curtman procedure has also this serious disadvantage (2). There is no serious loss, however, by the Pittmann procedure. The loss of ions by the proposed modified procedure was now examined. Iron, nickel, barium, and magnesium were taken as representative of the metals of later groups, and the loss of these metals was investigated as follows:—One milligram of each ion was mixed separately with 140 mg. of phosphate, and the phosphate was removed by the proposed procedure. The filtrate was then tested for the ion that was present before removal of the phosphate.

The results showed that there is practically no loss of ions in removal by the proposed procedure. One milligram of each of the metallic ions, Fe, Ni, Ba, Mg, could easily be detected after removal of 140 mg. of phosphate.

Using the proposed procedure, the following mixtures of salts were analysed, and in each case the metallic ions were recognised :—(*a*) Potash alum and potassium phosphate; (*b*) ferric chloride and potassium phosphate; (*c*) chromium sulphate and potassium phosphate; (*d*) aluminium sulphate, ferrous sulphate, and potassium phosphate; (*e*) chromium sulphate, ferric chloride, and potassium phosphate; (*f*) aluminium sulphate, chromium chloride, and potassium phosphate; (*g*) ferric chloride, nickel sulphate, and potassium phosphate; (*h*) zinc carbonate and sodium phosphate; (*i*) barium chloride and potassium phosphate.

Summary.

The Pittmann procedure for the removal of phosphate by means of zirconium oxychloride has been examined. It has been found to have many disadvantages. The causes of these have been studied, and a new procedure has been proposed in which the difficulties of the Pittmann procedure are overcome. It has been shown that by this proposed procedure the phosphate is completely removed in an easily filterable form, with practically no loss of other ions. The technique is simple. Small volumes are involved, which obviate tedious evaporation after removal. The very slight excess of reagent by this procedure does not interfere in the detection of the metals of later groups, and can be ignored. However, a scheme is suggested by which it can be isolated from the iron-aluminium group, if desired. As a rule the whole operation of removal can be carried out in less than 15 minutes, whereas by the Pittmann procedure it takes approximately 2 hours. From the standpoint of ease of technique and rapidity of execution, the proposed procedure is an advance on the methods used heretofore.

REFERENCES.

(1) Curtman and Greenslade.—J. Chem. Education, **13**, 5, 238 (1936).
(2) Pittmann, F. K.—Industrial and Engineering Chemistry (Analytical Ed.). Sept., 1940, p. 514.
(3) Curtman, Margulies, and Plechner.—Chem. News, **129**, 299 (1924).
(4) Bell, R. B., and Doisy, E. A.—J. Biol. Chem., 1920, **44**, 55.
Briggs, A. P.—J. Biol. Chem., 1922, **53**, 13.
Briggs, A. P.—J. Biol. Chem., 1924, **59**, 255.

No. 48.

NOTE ON THE ANALYSIS OF AN IRISH PHOSPHATE ORE.

By C. L. SIMPSON, M.Sc.,

AND

J. REILLY, M.A., D.Sc., F.I.C.

[Read November 25, 1941. Published separately February 11, 1942.]

A phosphate deposit occurs in a river valley near Doolin, in the west of County Clare, in what is classified, according to the general geological maps, as the limestone area. It is a hard rock phosphate, and from its mode of occurrence it may be classified as a sedimentary deposit formed by replacement of limestone. It is dark grey, almost black in appearance, and occurs as a fairly uniform bed 2 ft. 6 in. to 3 ft. in thickness, with an overburden of variable composition. This overburden consists in some places of light top-soil with 5 ft. to 7 ft. of shale, and in others of 5 ft. to 7 ft. of soil and clay with 2 ft. 9 in. of shale. Below the phosphate bed comes a further bed of shale 2 ft. 9 in. in thickness which is underlain by limestone.

A natural phosphate is of importance as a potential source of raw material for the manufacture of superphosphate to be used as a fertiliser. The chief constituent of such a phosphate is phosphoric acid (P_2O_5) or tribasic phosphate of lime ($Ca_3P_2O_8$), which is the form in which it generally occurs. Unfortunately, other constituents are almost invariably present, and these may be either diluents or actively disadvantageous, either in interfering with the manufacture of the superphosphate, or in lowering the quality of the product obtained.

Let us consider in order of importance the determination of the various constituents in a sample of the ore under investigation.

Phosphoric Acid (P_2O_5). The simplest method used for the estimation of total phosphate is the citro-magnesia method, which is purely gravimetric. This method is favoured in spite of its admitted defects because of its ease of manipulation, and also as it is comparatively speedy. It consists of precipitation of the phosphate as magnesium ammonium phosphate ($Mg . NH_4 . PO_4$) in the presence of ammonium citrate solution, which inhibits the co-precipitation of iron and alumina with the phosphate, and keeps the calcium in solution.

In other methods the phosphate is first precipitated as phospho-molybdate, and estimated, either volumetrically with standard alkali, or gravimetrically as magnesium ammonium phosphate, which on ignition is converted to magnesium pyrophosphate ($Mg_2P_2O_7$). The principle of these latter methods is the same, although the conditions of precipitation vary for different methods.

In his publication—"Analyse des Phosphates Naturels"—Paul Gavelle gives a comparative study of the citro-magnesia method and Jorgensen's phospho-molybdate method (1), and applies the two to the analysis of a Moroccan phosphate, and also to the analysis of a solution containing a known quantity of P_2O_5. The conclusions drawn from this comparative study are as follows:—

1. The citro-magnesia method applied to the analysis of phosphate cannot furnish, except accidentally, a strictly accurate content of P_2O_5. It admits of two causes of error in opposite directions:

(*a*) The solubility of ammonium magnesium phosphate in citrate of ammonia tends to make the results in P_2O_5 too low.

(*b*) The carrying off of lime in ammonium magnesium phosphate tends to produce too high results in P_2O_5.

Generally these two errors do not counterbalance each other. The extent of these errors varies, not only according to the composition of the phosphate analysed, but also according to the details of the mode of operation, which always varies from one chemist to another.

When this method is correctly followed the total of the two errors is slight, being about $+0{\cdot}3$ to $0{\cdot}4$ in the determination of P_2O_5 in a phosphate containing about 34 per cent. of P_2O_5, such as Morocco phosphate. This method, although sufficient for a manufacturer's purpose, cannot be accepted in the case of arbitrational analysis.

(2) The Jorgensen method, correctly carried out, gives contents of P_2O_5 which are scientifically accurate. The method of precipitation of the phospho-molybdate of ammonia prescribed by the author might with advantage be slightly modified. In our opinion the mineral solution should be heated to 50° C. before the solution of molybdate of ammonia is added.

Lundell and Hoffman in 1937 accepted the citro-magnesia method provided that two precipitations were effected. In estimating the P_2O_5 content of the sample, both the citro-magnesia method and Jorgensen's phospho-molybdate method were used.

The following are details of the citro-magnesia method in general use:—

Weigh 5 g. of the dried finely-ground sample into a 350 c.c. conical flask fitted with a small funnel (to prevent spitting). Add 50 c.c. of concentrated hydrochloric acid and 5 c.c. of concentrated nitric acid, and when effervescence ceases, boil gently for 30 min. Dilute with distilled water and filter into a 500 c.c. graduated flask, wash flask and filter well with hot distilled water. Cool, and make up to the mark with distilled water. Take 100 c.c. (equivalent to 1 g. of phosphate) for each estimation. Add ammonium hydroxide until a precipitate appears, and add immediately 75–125 c.c. of ammonium citrate solution according to the phosphate under examination. Next add 75–125 c.c. of magnesia mixture slowly and with constant stirring. Place on stirring machine, stir for half an hour, and allow to stand overnight. Filter through platinum gooch crucibles packed with asbestos, washing precipitate well with

3 per cent. ammonia solution until free from chlorides, and after drying ignite in a muffle furnace at 950–1000° C. The precipitate is weighed as $Mg_2P_2O_7$, and calculated as P_2O_5 or $Ca_3(PO_4)_2$ as required.

	Weight of $Mg_2P_2O_7$.	P_2O_5 Per cent.	$Ca_3(PO_4)_2$ Per cent.
Jorgensen Method.	0·185	23·60	51·56
	0·184	23·46	51·28
	0·185	23·60	51·56
	0·184	23·46	51·28
	Average	23·53	51·42
Citro-magnesia Method.	0·372	23·72	51·82
	0·373	23·79	51·96
	0·371	23·66	51·68
	0·372	23·72	51·82
	Average	23·72	51·82

Iron and Alumina. The iron in Clare phosphate is present in at least two forms, as oxide combined with the phosphate, and as sulphide (pyrites), the latter occurring as sporadic particles of varying size, some visible to the naked eye, others visible only under the microscope. There is also a further small amount of iron insoluble in acid, and soluble only on fusion, but this is an almost negligible quantity.

It is impossible to reproduce in the laboratory the actual conditions of mixing which occur in the manufacture of superphosphate with sulphuric acid as the solvent; consequently it is difficult to devise an absolute laboratory method for determining the amounts of iron and alumina which will react during the mixing, i.e. soluble iron and alumina. The most important factor governing these estimations seems to be the choice of a suitable acid solvent.

Lundell and Hoffman (2) recommend attack with constant boiling hydrochloric acid (1 : 1), and regard nitric acid as an unsatisfactory solvent, as it "has the disadvantage of dissolving pyrite, which is not wanted, and leaving haematite, which is." Aqua regia usually causes practically complete solution, and undoubtedly gives more "active iron and alumina, particularly iron, than is fair." In their routine method the iron and alumina are precipitated together as phosphates, the iron content is determined separately, and the alumina estimated by difference. By a second method (3) devised by the same analysts iron and calcium are removed before aluminium is precipitated as phosphate.

Owing to the variable results obtained for soluble alumina by these latter methods, concentrated hydrochloric acid was finally chosen as solvent for the phosphate. The iron and alumina were precipitated combined as phosphates

at pH (3·2–3·4) after the method of G. J. Austin (4). Therefore the results given for "soluble" iron and alumina are the amounts of these constituents soluble in concentrated hydrochloric acid, which acid is particularly suited to Austin's method of estimation.

For each estimation 5 g. of the sample were evaporated to dryness with 50 c.c. of concentrated hydrochloric acid, the residue taken up with 25 c.c. of the same acid, diluted to 100 c.c. with hot distilled water, and heated on the water-bath for 15 min., then filtered into a graduated 500 c.c. flask, the residue being washed well with hot distilled water. The flask and contents were cooled to room temperature, and the volume adjusted to the 500 c.c. graduation with distilled water.

The soluble iron is determined in 100 c.c. of the solution (representing 1 g. of the phosphate), using the method given in the Bureau of Standards pamphlet referred to previously. The iron and alumina are estimated together as phosphates, as previously stated, and the alumina determined by difference.

Using this method it was found that the phosphates were very easily filtered without the aid of filter-paper pulp, which on ignition tends to reduce the phosphates. Zinc, which appears to be the chief interfering element, was present in such small quantities that it was deemed unnecessary to reprecipitate the phosphates to avoid their contamination by this element.

Austin recommends the use of hot N/100 acetic acid as a wash solution, as "washing with ammonium acetate or nitrate solution tends to give low results owing to hydrolysis, whilst washing with ammonium phosphate solution tends to give too high results owing to adsorption of P_2O_5."

Results obtained:—

1st titration	... 4·95 c.c. $KMnO_4$	= 0·0123 g. Fe_2O_3	}	Mean = 0·0121 g.
2nd „	... 4·85 „ „	= 0·0120 „ „		
3rd „	... 4·90 „ „	= 0·0121 „ „		
4th „	... 4·90 „ „	= 0·0121 „ „		

(1 c.c. KMnO solution = 0·00247 g. Fe_2O_3 or 0·00467 g. $FePO_4$.)

Soluble Iron = 1·21 per cent. Fe_2O_3.

Weights of combined iron and alumina phosphates.

No. 1 = 0·041 g.	}	Mean = 0·0405 g.
No. 2 = 0·040 „		
No. 3 = 0·042 „		
No. 4 = 0·039 „		

($AlPO_4 \times 0·418 = Al_2O_3$.)

Weight of $FePO_4$ equivalent to 0·0121 g. Fe_2O_3 = 0·023 g.
Weight of $AlPO_4$ = (0·0405–0·023) = 0·0195 g., equivalent to 0·0082 g. Al_2O_3.
Soluble Alumina = 0·82 per cent. Al_2O_3.

The figure obtained for soluble iron, using concentrated hydrochloric acid as solvent, agreed almost exactly with that obtained by solution in constant boiling hydrochloric acid, and also with that obtained when the mixed phosphates were dissolved in hydrochloric acid, and the iron was determined volumetrically.

The residue insoluble in concentrated hydrochloric acid on fusion with (Na_2CO_3–K_2CO_3) yielded an amount of iron equivalent to 1·85 per cent. Fe_2O_3. This includes the iron which is insoluble in aqua regia (0·07 per cent. Fe_2O_3). Addition of the two amounts, soluble and insoluble, gives a figure for total iron equal to 3·06 per cent. Fe_2O_3. The insoluble residue also yielded 0·18 per cent. Al_2O_3, and was estimated in a manner similar to that used for soluble alumina, with the addition of 5 c.c. of molar potassium dihydrogen phosphate at the stage indicated.

Results obtained:—

1st titration	... 7·6 c.c. $KMnO_4$	= 0·0187 g. Fe_2O_3	}	Mean = 0·0185 g.
2nd „	... 7·4 „ „	= 0·0183 „ „		
3rd „	... 7·5 „ „	= 0·0185 „ „		
4th „	... 7·5 „ „	= 0·0185 „ „		

Insoluble Iron = 1·85 per cent. Fe_2O_3.

Weights of combined iron and alumina phosphate.

No. 1 = 0·040 g.	}	Mean = 0·0395 g.
No. 2 = 0·038 „		
No. 3 = 0·041 „		
No. 4 = 0·039 „		

Weight of $FePO_4$ equivalent to 0·0185 g. Fe_2O_3 = 0·035 g.
Weight of $AlPO_4$ = (0·0395 – 0·035) = 0·0045 g.
Weight of Al_2O_3 = (0·0045 × 0·418) = 0·0018 g.

Insoluble Alumina = 0·18 per cent. Al_2O_3.

Total Iron and Alumina. The following method was used:—After evaporation to dryness of 5 g. of the sample with concentrated hydrochloric acid, as for soluble iron and alumina, take up with the same acid, and filter. Ignite residue, and fuse with (Na_2CO_3–K_2CO_3), take up the melt with dilute hydrochloric acid, filter, and wash well, adding the washings to the first filtrate. Remove silica by two evaporations to dryness with hydrochloric acid, take up the residue with 25 c.c. of concentrated hydrochloric acid, and proceed as for soluble iron and alumina.

The iron is determined in 100 c.c. portions of the solution, but only a 50 c.c. aliquot is taken for the alumina estimation, so that the weight of the combined phosphates approximates to that obtained for soluble iron and alumina.

Results obtained:—

1st titration	... 12·7 c.c. $KMnO_4$	= 0·031 g. Fe_2O_3	Mean = 0·031 g.
2nd „	... 12·5 „ „	= 0·031 „ „	
3rd „	... 12·65 „ „	= 0·031 „ „	
4th „	... 12·55 „ „	= 0·031 „ „	

Total Iron = 3·10 per cent. Fe_2O_3.

Weights of combined iron and alumina phosphates.

(50 c.c. aliquots = 0·5 g. of phosphate.)

No. 1 = 0·040 g.
No. 2 = 0·044 „
No. 3 = 0·041 „
No. 4 = 0·043 „
} Mean = 0·042 g.

Weight of $FePO_4$ equivalent to 0·031 g. Fe_2O_3 = 0·059 g.

Weight of $AlPO_4$ = (0·084–0·059) = 0·025 g. equivalent to 0·0105 g. Al_2O_3.

Total Alumina = 1·05 per cent. Al_2O_3.

The results obtained by addition of the quantities obtained by estimation in the soluble and insoluble portions are

3·06 per cent. Fe_2O_3 and 1·10 per cent. Al_2O_3.

Iron Sulphide (FeS_2). Deducting from the values obtained for total iron the amounts soluble in hydrochloric acid and insoluble in aqua regia, the residual percentage of iron is 1·81 per cent. Fe_2O_3, which is equivalent to 2·72 per cent. FeS_2.

Separate 1 g. portions of the phosphate were dissolved in aqua regia, and the sulphur estimated as barium sulphate in the usual fashion. After deduction of the amount of sulphur soluble in hydrochloric acid (0·075 per cent.), this gave an amount of sulphur equivalent to 2·69 per cent. FeS_2. These results agree pretty well with the figure (2·67 per cent. FeS_2) obtained by estimating the iron soluble in aqua regia after ignition of the original hydrochloric acid insoluble residue.

Carbonates. The carbonic acid content may be conveniently determined by difference in Schrotter's apparatus, using dilute hydrochloric acid to decompose the carbonates. Using this method it is difficult to be certain that all the carbon dioxide is driven off, and that nothing other than carbon dioxide has been lost. It is more accurate to collect the carbon dioxide by absorption in soda-lime, using the usual train of apparatus for drying and purifying the gas evolved. For each estimation 2 g. of the phosphate are placed in a decomposition flask and 100 c.c. of dilute hydrochloric acid added by means of a dropping funnel. After primary decomposition ceases the flask is heated just

to boiling point, while a slow stream of carbon-dioxide-free air is drawn through the apparatus.

Weights of Absorption Tube.		Weight of Carbon Dioxide absorbed.
Before.	After.	
51·678 g.	51·913 g.	0·235 g.
55·289 „	55·525 „	0·236 „
51·913 „	56·148 „	0·235 „
55·525 „	55·759 „	0·234 „
	Mean Weight	0·235 „

Carbon Dioxide = 11·75 per cent.

Fluoride. Fluorine is usually estimated by fusion of the phosphate with a mixture of sodium and potassium carbonates, and, after removal of interfering elements, by final precipitation as calcium fluoride or sulphate. The chief objection to final estimation as calcium fluoride appears to be the solubility of calcium fluoride in water, and the difficulty in filtering the precipitate. The fluorine is preferably precipitated as lead chlorofluoride (5), which has a much higher molecular weight than calcium fluoride. Errors due to solubility of the lead chlorofluoride are greatly reduced by the use of a saturated solution of lead chlorofluoride for the greater part of the washing of the precipitate.

For each estimation 1 g. of phosphate is fused with K_2CO_3, the melt leached with hot water, filtered, and well washed. The last traces of silica are removed by the addition of zinc oxide in nitric acid (1 g. ZnO in 30 c.c. N. HNO_3). The fluoride is precipitated by the addition of a large excess of lead chloride and allowed to stand overnight. It is filtered through a weighed Gooch crucible, washed once with alcohol, then four or five times with saturated lead chlorofluoride solution, and finally with cold water, and dried at 120–150° C.

Weight of crucible.	Weight of crucible and precipitate.	Weight of lead chlorofluoride.
17·930 g.	18·210 g.	0·280 g.
18·680 „	18·950 „	0·270 „

Fluorine = 2 per cent.

Silica. In the estimation of this element the method used was that of Berzelius (6) modified by Hoffman and Lundell.

1 g. of the phosphate is intimately mixed with 5 g. of fusion mixture (Na_2CO_3–K_2CO_3) covered with a further 1 g. of the mixture, fused at dull red heat, and the melt extracted with hot water.

The silica is separated in three stages, first by boiling with 2 per cent. sodium carbonate solution. The silicious residue is retained, and the filtrate

treated with zinc nitrate (1 g. ZnO in 30 c.c. N. HNO_3), boiled, and filtered from a second silicious residue. The new filtrate is rendered slightly acid with nitric acid, and to it is added a reagent (made by dissolving 1 g. ZnO, 2 g. $(NH_4)_2CO_3$ in 20 c.c. H_2O containing 2 c.c. NH_4OH (conc)). The mixture is boiled free from ammonia, and the volume adjusted to about 100 c.c. The third silicious residue is filtered off, combined with the two previous ones, evaporated to dryness with hydrochloric acid, washed with water containing the same acid (20 : 1), ignited, and weighed.

Weights of SiO_2, 1st estimation = 0·108 g.
2nd „ = 0·107 „
Percentage of SiO_2 = 10·75 per cent.

Calcium. The solution for estimation of total lime is prepared as in the method for total iron and alumina given earlier in this paper—50 c.c. aliquots (equivalent to 0·5 g. of phosphate) are treated as in the method given in the Bureau of Standards leaflet (3).

Weights of CaO obtained :—

1st estimation = 0·226 g.
2nd „ = 0·225 „
3rd „ = 0·225 „
4th „ = 0·224 „
Percentage of lime (CaO) = 45·00.

"The method gives results that are comparable with those obtained by the ordinary oxalate method in rock analysis. Because of the solubility of calcium oxalate a little is unprecipitated in both methods, but the total amount need not exceed 0·5 mg. (0·1 per cent. CaO in a 0·5 g. sample in double precipitations). In the accurate analysis of minerals this is subsequently recovered by treatment of the weighed magnesium pyrophosphate; in the analysis of phosphate rock this refinement is not necessary.

The treatment with iron, ammonium hydroxide, and bromine serves quite satisfactorily for the removal of manganese and residual phosphoric acid, and numerous tests have shown that usually less than 0·2 mg. of CaO is carried down with the precipitate."

Zinc and Manganese. The solution for estimation is prepared as for total iron and alumina. The zinc is separated as sulphide at pH (4·6–4·8), and the manganese as oxide, using G. J. Austin's method of estimation (4). Aliquots equivalent to 2 g. of phosphate were used for each estimation, so that fairly appreciable weights of precipitate could be obtained. Both the sulphide and oxide are dissolved in the least possible quantity of 50 per cent. hydrochloric acid, precipitated as ammonium phosphates, and estimated finally as pyrophosphates (7).

Weights of zinc pyrophosphate.	Weight of zinc oxide.
0·015 g.	0·008 g.
0·011 „	0·006 „

(i.e. for 2 g. of phosphate.)

Percentage of ZnO = **0·35**.

Weights of manganese pyrophosphate.	Weight of MnO.
0·020 g.	0·010 g.
0·016 „	0·008 „

(i.e. for 2 g. of phosphate.)

Percentage of MnO = **0·45**.

Iodine. Two methods were used for the estimation of iodine. The first is that (8) in present use by Dr. Mason at University College, Cork, and is known as the "open-ashing" method. The second method is the Trevorrow and Fashena modification of Leipert's technique (9). In both these methods the iodine is estimated finally by titration with N/500 sodium thiosulphate, using starch as indicator, and the reagents are tested for freedom from iodine by running "blank" estimations in parallel.

Duplicate results were :—

"Open-ashing" Method.	Acid "wet combustion" Method.
100 γ per 100 g.	174 γ per 100 g.
	310 γ „ „ „

The results given for the second method are the most reasonable of four estimations, the other two results being excessive, probably due to adsorption of iodine in the laboratory. The results obtained by the first method being the more reliable are given in the table.

Arsenic. Arsenic is estimated by the usual Marsh method, by comparison of the stain produced with a standard. Two estimations were made, of which the mean result was 0·0001 per cent.

Organic Matter. Although this constituent was not determined in the particular sample analysed, it is to be expected that such a deposit should contain a certain amount of organic matter. In the absence of other metallic and acidic radicals, the amount of organic matter present may be taken as being equivalent to the undetermined portion of the analysis.

In all, twelve samples of the same deposit have been analysed for calcium phosphate content. The several results obtained varied from 49·32 per cent. to 51·90 per cent., and indicate comparative uniformity of the deposit. The table shows complete analysis of one such sample.

This was a macro-analysis, the micro-estimation of iodine and arsenic being introduced merely as points of interest. The sample was ground to pass completely through a 90 I.M.M. sieve, and dried for three hours at 100° C.

		Per cent.
Phosphoric Acid	(P_2O_5)	23·53 equivalent to 51·42% $Ca_3P_2O_8$.
Lime	(CaO)	45·00
Iron Oxide	(Fe_2O_3)	1·28
Alumina	(Al_2O_3)	1·05
Pyrites	(FeS_2)	2·70
Silica	(SiO_2)	10·75
Carbonic Acid	(CO_2)	11·75
Fluorine ...	...	2·00
Zinc	(ZnO)	0·35
Manganese	(MnO)	0·45
Nitrogen ...	...	0·22
Sulphuric Acid	(SO_3)	0·20
Undetermined		0·72
	Total	100·00
Loss on Ignition	...	10·65
Soluble Iron ...	...	1·21
Soluble Alumina	...	0·82
Arsenic ...	...	0·0001
Iodine ...	...	100 γ per 100 g.

This ore does not readily respond to treatment with sulphuric acid, and it has not been possible to render it as completely soluble as desired. On treatment with less than the theoretical amount of acid required, a low grade superphosphate with a high percentage of insoluble phosphate and of only moderate drillability is obtained.

The only minor constituents present in excessive quantities in the ore are carbonate and silica, and, chemically, these should not cause serious drawbacks. A feasible theory for the failure to render this phosphate soluble in a satisfactory manner is that these minor constituents are so distributed through the mass of the phosphate as to form a protective sheath over the tricalcium phosphate and to prevent the sulphuric acid from interacting with it.

It would not be possible in such a paper as this to consider the various reactions incidental to the manufacture of superphosphate, and for details of these reactions reference should be made to specialized literature on the subject. It is sufficient to state that the primary reaction is between the tricalcium phosphate and sulphuric acid with the formation of monocalcium phosphate and gypsum, and that the degree of solubility of the phosphate, and its

drillability, depend to a large extent on the amounts of the minor constituents present and the forms in which they occur.

CHEMICAL DEPARTMENT,
UNIVERSITY COLLEGE,
CORK.

REFERENCES.

1. "Determination of Phosphoric Acid in Mineral Phosphates and Phosphatic Fertilisers," by Gunner Jorgensen of Copenhagen.
2. "The Analysis of Phosphate Rock," by G. E. F. Lundell and J. I. Hoffman, in Journal of the Association of Official Agricultural Chemists, VIII, No. 2. 15th November, 1924.
3. Bureau of Standards Certificate of Analyses of Standard Sample, No. 56. (Tennessee Rock Phosphate.)
4. A New Group Separation for the Quantitative and Qualitative Analysis of Phosphates, by G. J. Austin. (*Analyst*, June, 1940.)
5. Thorpe's Dictionary of Applied Chemistry, 4th edition, Vol. 2.
6. Berzelius Method modified by Hoffman and Lundell. (Bur. Stand. J. Res., 1929, 3, 381.)
7. Same as 5.
8. The Determination of Iodine in Biol. Substances, by C. O. Harvey. (M.R.C. Spec. Report Series, No. 201, 1935.)
9. Trevorrow and Fashena.—J. Biol. Chem., 1935, **110**, 29.
   Fashena and Trevorrow.—J. Biol. Chem., 1936, **114**, 351.
   Leipert.—Biochem. Ztschr., 1933, **261**, 436.

No. 49.

SALMON OF THE RIVER ERNE.

RESULTS OF THE EXAMINATION OF A SMALL COLLECTION OF SCALES AND DATA.

By ARTHUR E. J. WENT,

Department of Agriculture, Fisheries Branch, Dublin.

[Read December 16, 1941. Published separately March 5, 1942.]

With a valuable fish like the salmon it is not possible to purchase large samples for examination in the laboratory, as can be done with the herring and other less expensive fish. Recourse has, therefore, to be had to other methods of obtaining material. It has been found satisfactory to arrange with the manager of a commercial salmon fishery on the river which it is desired to investigate to collect samples of scales, together with particulars of the measurements (weight to nearest 2 oz., length to nearest 1/10th inch), the sex, the place, and date of capture. Over a number of years collections of scales and measurements of salmon taken in various rivers throughout Ireland have been made with the intention of obtaining information on the age and growth of salmon of these rivers. A small collection of material was available from the River Erne, and, although it was obviously inadequate in many respects, it was considered of sufficient interest to be worked out and reported upon.

The proportion of fish sampled showed some fluctuation over the season, and in most cases the results have been weighted against the catch figures for 1928 in order that a more accurate idea of the stocks of the river could be obtained. The weighted figures have been given in Tables 1–4 and in suitable places in the text.

The River Erne and its Catchment Area.

The River Erne is 64 miles long, and the catchment area of the river and its tributaries covers approximately 1,690 square miles. There is a large number of lakes in this system, the most important of which are Lough Erne, Upper Lough Erne, Loughs Oughter and Gowna, all on the main river, and Loughs MacNean, Nilly, and Garadice, on the tributaries. The river rises in County Longford, flows for the major part of its length in a northerly or north-westerly direction, and finally empties into an estuary, about four miles long, which is an inlet of Donegal Bay. In the upper part of the catchment area there are hundreds of small lakes which contain coarse fish only. Salmon seldom, if ever, penetrate into them.

The main river from Lough Oughter, and the main tributaries flowing into the main watercourse from the west, run over rocks of the Lower Carboniferous Limestone, and in consequence these waters are alkaline in character. The Annalee River, the most important tributary of the River Erne, and the River Erne itself above Lough Oughter, drain areas of rocks of the Silurian and Ordivician periods, which contain little lime, and thence the waters of these rivers are neutral or slightly acid in character. On the east side of Lough Erne a number of small tributaries run over rocks of the Old Red Sandstone,

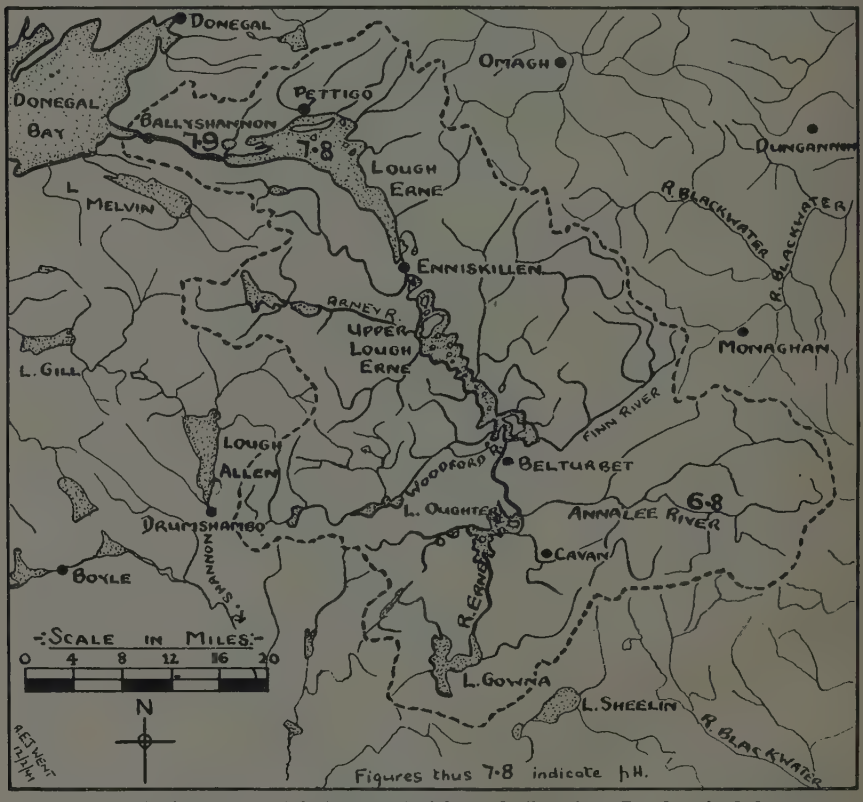

Fig. 1.—Catchment area of the R. Erne, its lakes and tributaries. (Based on the Ordnance Survey Map of Northern Ireland, by sanction of the Controller of H.M. Stationery Office and the Ordnance Survey of Eire, by permission of the Minister for Finance of Ireland.)

and in consequence these waters are acid in character. The relationship of the River Erne to its lakes and tributaries is represented in the form of a sketch-map in Fig. 1. On this figure a few pH figures have been inserted for the waters of particular localities.

Material.

The material used in this investigation consists of 421 satisfactory sets of scales and data collected from salmon captured in the tidal nets and the cribs below the bridge in the town of Ballyshannon, Co. Donegal, in the river in 1928, together with a few sets from the years 1921, 1926, and 1927. I am indebted to Mr. Thomas McD. Swan, of the Erne Fisheries, for collecting the material and furnishing me with confidential returns of the fishery for 1928. All the fish were measured from the tip of the snout to the fork of the tail to the nearest tenth of an inch.

Smolt Ages.

In the maiden fish, 346 fish (92·0 per cent.) migrated as two-year smolts, 18 fish (4·8 per cent.) as one-year smolts, and 12 fish (3·2 per cent.) as three-year smolts.

Age Groups.

The fish were classified into five groups of maiden or unspawned fish, and two groups of fish containing those which had previously spawned (i.e. with spawning marks on their scales). In the following table the fish are divided into their respective age groups:—

Winters in the sea.	1+	2	2+	3	3+	With one S.M.	With two S.M.s
% of total	58·7	7·5	25·2	1·7	0·5	6·2	0·2

The maiden summer fish (1+, 2+, and 3+ winters) form 84·4 per cent. of the total numerically. Commercially the small summer fish, having relatively high average weights, form the most important age group, being approximately one-sixth heavier than the grilse, the next heaviest age group.

Confirmation of the fact that summer fish form a considerable part of the runs of salmon in this river can be obtained by consideration of the distribution in time of the catches of salmon (and grilse). In Fig. 2, which has been plotted from confidential figures kindly supplied by Mr. Thomas McD. Swan, the fortnightly percentages of the total catch for the year 1928 have been given. That similar conditions existed in other years can be shown by the fact that the appropriate curve for the year 1940,[1] which has been taken at random, shows a similar result.

[1] Up to and including the year 1933 the Erne Fisheries operated two draft nets in the tidal portion of the river and a set of cribs above the Falls of Assaroe in the town of Ballyshannon. In consequence of the judgment in the "Erne Case" in which it was decided that no several fishery existed in the tidal portion of the river, the fishery was thrown open to the public. From 1934 onwards a special local licence costing £40 for each draft net has been obtainable for fishing this river under the authority of the Fisheries (Tidal Waters) Act, 1934. Each licensee is bound to furnish a weekly return of his catches, and the curve for 1940 has been drawn up from the weekly returns of some thirteen licensees for that year. Since 1933 the Erne Fisheries have continued to operate the set of traps near the Falls of Assaroe.

As is usually the case, very few summer fish were captured in April; in May they formed just under half of the total catch, and thereafter they predominated (Table 1). May was the best month for the small spring fish, June for the grilse, small summer, and large spring fish, whilst most previously spawned fish were taken in July. The catches in June amounted to 64·6 per cent. of the total for 1928, and those of July to 24·0 per cent. (Table 2).

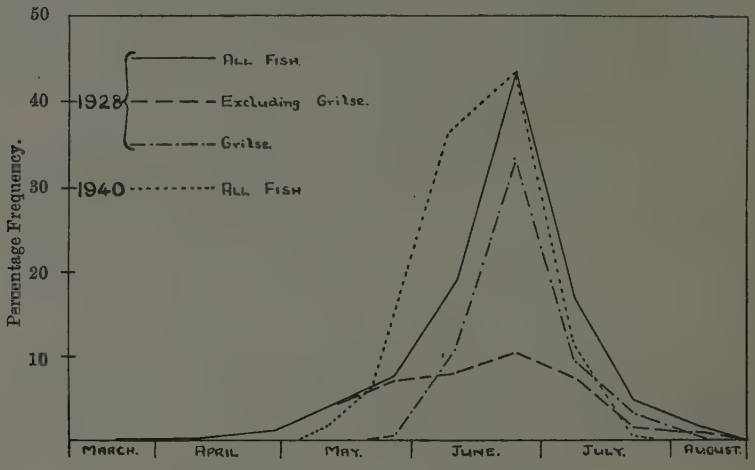

Fig. 2—Distribution in time of the catches.

Forty-five fish, out of the total of 421 whose scales were examined, had spawned previously, as indicated by the presence of spawning marks on their scales. Two of these previously spawned fish had each spawned on two occasions, and were making their way into the river in anticipation of a third spawning. The determination of the age at first spawning is liable to some inaccuracy after the formation of the spawning mark and the return to the river as a clean fish, as erosion may remove traces of winter and even of summer growth. In Table 3 the age at first spawning has been correlated with the absence, which is the period spent feeding in the sea between what would be, but for the intervention of man, two successive spawnings. Owing to the difficulty of assessing the age at first spawning the figures in the table must be regarded as approximate. The summer fish generally show a tendency to return after a short or very long absence, while the spring fish return after a long absence. This is the condition which has been found in other investigations on the life history of salmon.

Size Distribution.

In Table 4 the estimated size distributions in each age group, as percentages of the total catch, have been given for two-inch class intervals. The curve

Table 1 Estimated Percentage of each age group in each months's catch.

Period	Age Group						Total
	1+	2	2+	3	3+	With S.M.s	
22nd March to 30th April	-	48.0	7.7	21.2	-	23.1	100.0
May	2.8	32.3	43.1	7.0	-	14.8	100.0
June	68.7	4.4	22.9	1.3	0.2	2.5	100.0
1st July to 11th August	58.3	3.6	23.6	-	1.8	12.7	100.0
Total	58.7	7.5	25.2	1.7	0.5	6.4	100.0

Table 2 Estimated Percentage of total of each age group in each month.

Period	Age Group						Total
	1+	2	2+	3	3+	With S.M.s	
22nd March to 30th April	-	3.4	0.2	6.5	-	1.9	0.5
May	0.5	47.1	18.7	44.3	-	25.3	10.9
June	75.7	37.9	58.7	49.2	22.0	25.6	64.6
1st July to 11th August	23.8	11.6	22.4	-	78.0	47.2	24.0
Total	100.0	100.0	100.0	100.0	100.0	100.0	100.0

x For convenience the period 22nd March to 31st March has been included with April and the period 1st-11th August has been included with July in Tables 1 and 2.

Table 3 Previously spawned fish classified on absence.

Absence	Age at first spawning			Total
	1+ Winters	2 Winters	2+ Winters	
Short (less than complete year)	7	-	3	10
Long (complete year)	-	17	4	21
Very long (more than complete year)	6	-	6	12

Table 4 Frequency distribution of sizes in the different age groups.

Class Interval xx	Age Group						Total
	1+	2	2+	3	3+	With S.M.s	
16	1.2	-	-	-	-	-	1.2
18	0.6	-	-	-	-	-	0.6
20	9.4	-	-	-	-	-	9.4
22	25.8	-	-	-	-	-	25.8
24	18.8	0.1	-	-	-	0.1	19.0
26	2.9	0.4	-	-	-	0.2	3.5
28	-	2.0	1.5	-	-	0.4	3.9
30	-	3.0	9.2	-	-	0.6	12.8
32	-	1.6	9.6	-	-	0.6	11.8
34	-	0.4	3.6	0.2	-	1.3	5.5
36	-	-	1.0	0.4	0.5	1.6	3.5
38	-	-	0.3	0.3	-	1.3	1.9
40	-	-	-	0.4	-	0.2	0.6
42	-	-	-	0.4	-	0.1	0.5
Total	58.7	7.5	25.2	1.7	0.5	6.4	100.0

xx Class interval 16 etc. includes all fish having lengths between 15.95 and 17. 95 inches etc.

Table 5 Mean Condition Co-efficient (K) and Condition Factors (according to Corbett's scale) (C.F.) of the different age groups.

Age Group	Condition Co-efficient (K)	Condition Factor (C.F.)
1+ winters	1.17	42.2
2 winters	1.16	42.3
2+ winters	1.21	43.7
3 winters	1.25	44.8
3+ winters	1.11 (one only)	40.0
With S.M.s.	1.21	43.7
Total	1.18	42.6

Table 6 Showing divided migration and return. The following table gives the years in which the fish examined were hatched in percentages of the total catch for the year 1928.

Returned in 1928 as	Hatched in the year						Total
	1920	1922	1923	1924	1925	1926	
Grilse (1+ Winters)	-	-	-	1.7	55.8	1.2	58.7
Small Spring fish (2 Winters)	-	-	0.3	6.7	0.5	-	7.5
Small Summer fish (2+ Winters)	-	-	0.8	23.2	1.2	-	25.2
Large Spring fish (3 Winters)	-	0.1	1.5	0.1	-	-	1.7
Large Summer fish (3+ Winters)	-	-	0.5	-	-	-	0.5
With S.M.s †	0.1	4.1	1.2	0.9	0.1	0.1	6.4
Total	0.1	4.2	4.3	32.6	57.6	1.2	100.0

† Approximate values.

Table 7 Mean calculated lengths in inches in fresh water.

Smolt Age	Numbers	Lengths at end of			Mean Smolt Length
		1st Year	2nd Year	3rd Year	
1	18	3.1	-	-	5.2
2	332	2.1	5.0	-	5.6
3	12	2.0	4.3	5.8	5.8

Table 8 Estimated proportion of the different smolt types in each smolt class for maiden fish

Smolt Age	Type A		Type B	
	Number	Percentage	Number	Percentage
1	-	-	18	4.8
2	57	15.1	289	76.9
3	11	2.9	1	0.3

Table 9 Mean calculated lengths in inches in the sea in the various age groups.

Age Group	Numbers	Lengths at end of		
		1st Sea Winter	2nd Sea Winter	3rd Sea Winter
1+ Winters	100	18.3	-	-
2 Winters	87	19.0	30.9	-
2+ Winters	152	18.6	31.2	-
3 Winters	21	19.7	31.9	39.3

Table 10 Mean calculated lengths in inches in fresh water in the various smolt classes and types.

Smolt Age	Type A Smolts					Type B Smolts				
	Numbers	Lengths at end of			Smolt Lengths	Numbers	Lengths at end of			Smolt Lengths
		1st Year	2nd Year	3rd Year			1st Year	2nd Year	3rd Year	
1	-	-	-	-	-	18	3.1	-	-	5.2
2	57	2.3	5.7	-	5.7	289	2.0	4.8	-	5.5
3	11	2.0	4.3	5.8	5.8	1	1.7	4.0	5.3	5.8

showing the size distributions has two maxima at 22 and 30 inches, respectively, which are produced by the overwhelming abundance of the grilse and small summer fish, as can be seen from Fig. 3. Fish, the lengths of which were between 19·95 and 25·95 inches, comprised slightly more than half the total catch, whilst fish having lengths between 29·95 and 33·95 inches formed about one-quarter of the total catch.

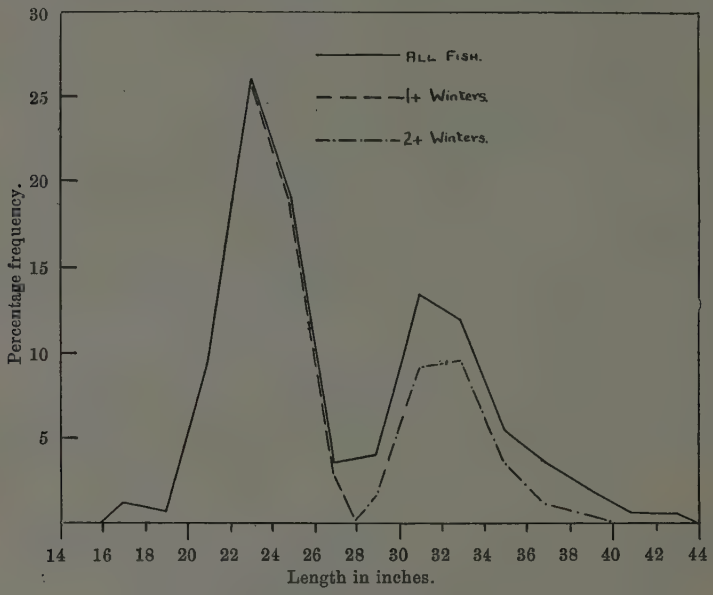

Fig. 3.—Estimated size distribution of the catches.

Average Sizes.

With a small collection of sets of scales and data such as were used in this investigation the monthly average weights and lengths of the different age groups are of little value, since in some cases only a few fish were sampled in any one month. In the following discussion the average sizes of the fish of the various age groups for the whole of the season have been given :—

(i) Grilse (1 + winters). Number examined = 105.

	lb.	inches.
Minimum	2·0	17·6
Average	5·4	23·2
Maximum	9·5	27·5

The smallest grilse was taken on the 31st May, together with another fish of the same length but weighing $\frac{1}{4}$ lb. more, whilst the maximum weight and length were recorded on the 11th July. Out of 105 grilse, 2 had migrated as

one-year smolts, 100 (95·2 per cent.) as two-year smolts, and 3 had migrated as three-year smolts.

(ii) Small spring fish (2 winters). Number examined = 88.

	lb.	inches.
Minimum	5·5	24·0
Average	12·5	30·9
Maximum	18·75	35·8

The smallest fish of this age group was taken on the 19th May, whilst the maximum weight and length were recorded on the 4th May. Out of the 88 small spring fish examined, 6 had migrated as one-year smolts, 78 (88·8 per cent.) as two-year smolts, and 4 as three-year smolts.

(iii) Small summer fish (2 + winters). Number examined = 161.

	lb.	inches.
Minimum	9·0	28·0
Average	14·7	32·5
Maximum	26·0	37·8
	23·25	38·2

The smallest fish was taken on the 19th May. A weight of 26 lb. was recorded twice on the same day, namely, 2nd June, 1927, both fish having the same length. A longer but lighter fish, weighing only 23·25 lb. (length 38·2 inches), was captured on 24th May. Out of the 161 fish whose scales were examined, 9 had migrated as one-year smolts, 148 (91·8 per cent.) as two-year smolts, and 4 as three-year smolts.

(iv) Large spring fish (3 winters). Number examined = 21.

	lb.	inches.
Minimum	16·5	34·4
Average	27·9	39·3
Maximum	40·0	43·7

The smallest fish was taken on the 22nd May, and on the 31st May the largest fish was recorded. Out of the 21 sets of scales examined, one was taken from a fish which had migrated as a one-year smolt, 19 as two-year smolts, and one had migrated as a three-year smolt.

(v) Large summer fish (3 + winters).

Only one fish, weighing 21 lb., measuring 37·4 inches in length, and captured on the 4th June, was examined in this age group.

(vi) Previously spawned fish (with S.M.s). Number examined = 45.

This group comprises a heterogeneous collection of individuals having only

one common property, namely, the presence of one or more spawning marks on their scales.

		lb.	inches.
Minimum	... ...	7·0	24·0
Average	... ...	19·7	35·2
Maximum	... ...	33·0	42·3

The smallest fish, captured on the 6th July, had spawned for the first time as a grilse, and had returned to fresh water for the second time after a period feeding in the sea of only a few months. The largest fish was captured on the 9th May, and showed two spawning marks on its scales. Average weights and length in the case of previously spawned fish are, however, of little value, as the weight of a previously spawned fish will depend on at least three factors, namely, the age at first spawning, the period spent feeding in the sea between successive spawnings (absence habit), and the number of times a fish had spawned. It is usual to classify the previously spawned fish on their absence habit and the age at first spawning, but in this investigation the numbers of fish in this category (i.e. with S.M.s) are too few to give significant results.

Condition Coefficients.

In a previous publication dealing with the salmon of the Ballisodare River (Went, 1941) the method of calculating the condition coefficient (K) and the condition factor (C.F.) or the relationship between weight and length was described. The mean condition coefficients (K) and condition factors (C.F.) have been given in Table 5. The mean condition coefficient rises as the time spent feeding in the sea increases. The high mean condition coefficient of the salmon of the River Erne is of the same order as that of the salmon of the Rivers Shannon and Ballisodare, although the small summer fish of the River Erne were superior in mean condition coefficients to those of the River Shannon.

Divided Migration and Return.

Table 6 gives the years in which the fish were hatched. The brood of one year, namely, that of 1925, was responsible for over half the catch in 1928, whilst the brood of 1924 accounted for one-third of the catch in 1928.

The Character of the Annuli in the Central Region of the Scales.

During the examination of the scales it became clear that the fish could be divided into two fairly distinct groups depending on the appearance of the annuli in the central region of the scale corresponding to freshwater or parr growth. In 10 per cent. or thereabouts of the fish the winter band was poorly defined. These differences were probably the results of life in different environments, and seemed worthy of note.

Calculated Lengths.

The lengths of every maiden or unspawned fish were calculated for the end of every year of life by the usual method, i.e. assuming that the growth of the scale was strictly proportional to that of the fish.

(*a*) *River Life.*

In Table 7 the mean lengths in inches of the different smolt classes have been given, and these are illustrated graphically in Fig. 4A. It will be seen that the fastest growing smolts migrated first, and that the mean smolt length increased as the age of the smolt rose.

In previous publications on the biology of the salmon of the Rivers Shannon, Ballisodare, and Owenduff (Ballycroy), it was stated that the fish of these rivers could be divided into two smolt types, called for convenience, Type A and Type B smolts. These were (1) Type A smolts, those fish which showed little or no growth in fresh water in the spring prior to migration as a smolt, and (2) Type B smolts, those fish which showed considerable amount of growth in fresh water in the spring prior to migration as a smolt.

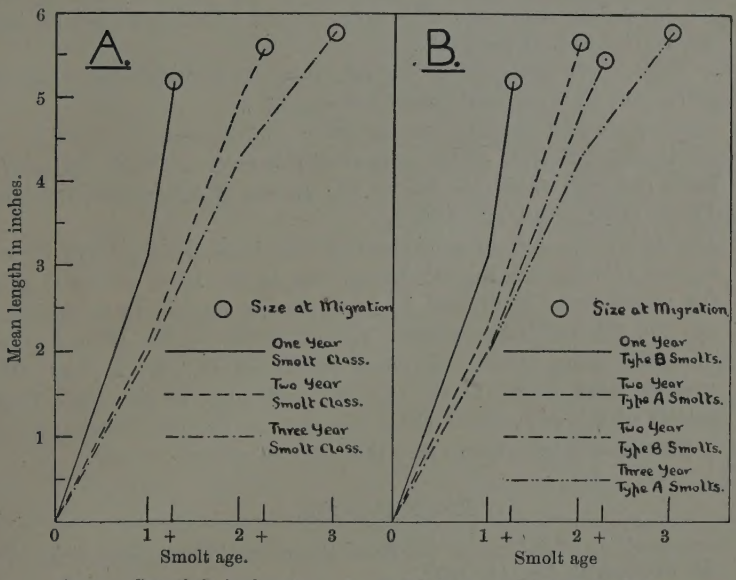

FIG. 4.—Growth in freshwater. A.—In the 1-, 2-, and 3-year smolt classes. B.—In the various smolt classes and types.

In the present material this division of the smolts into two groups was again carried out, and the results for the maiden fish are given in Table 8. The mean lengths in each of these groups have been calculated, and the results given in Table 10 are illustrated in Fig. 4B. In the two-year smolt class the

Type A smolts at the end of each year were longer than those of the Type B group. The material is too meagre, however, to draw any definite conclusions, but it can be noted that the results are of the same character as has already been described for other Irish rivers (Went, 1938, 1940, and 1941). The mean length of all the smolts was 5·6 inches, which is of the same order as that for the River Shannon (Went, 1940) and the Waterville River (unpublished data).

(*b*) *Sea Life.*

The calculated lengths for each year in the sea in each age group have been given in Table 9, and it will be seen that the growth rate in the large spring fish was greater than in any other age group.

Résumé.

(1) A brief description of the River Erne and the geology of its catchment area was given, Fig. 1.

(2) Material consisting of 421 satisfactory sets of scales from the River Erne nets and cribs was examined.

(3) Ninety-two per cent. of the fish had migrated as two-year smolts, whilst the remaining eight per cent. were one and three-year smolts in the ratios of 3 to 2 respectively.

(4) Almost eighty-four per cent. of the fish were summer fish (58·7 per cent. grilse and 25·2 per cent. small summer fish).

(5) The average sizes in the various age groups have been given. The mean condition coefficients of the salmon of this river are high, being of the same order as those for fish of the Rivers Shannon and Ballisodare (Went, 1938, 1940, and 1941).

(6) The lengths at the end of each year of life have been calculated. The fastest growing fish migrated first. As in the River Shannon salmon the smolts could be divided into two groups, depending on whether or not the fish had made growth in fresh water in the spring prior to migration as a smolt. Type A smolts of any smolt class have a greater mean length at the end of every year of life than Type B smolts of the same smolt class. In the sea the large spring fish had a greater average growth-rate than any other age group.

REFERENCES.

Went, Arthur E. J.—"Salmon of the River Shannon." Proc. Roy. Irish Acad., **44**, Section B, No. 11, 1938.

———"Salmon of the River Shannon." Journal of the Department of Agriculture, **37**, No. 2, September, 1940.

———"Salmon of the Ballisodare River, Part II. Age and Growth." Sci. Proc. R. Dublin Soc., **22** (N.S.), No. 35, January, 1941.

———"Salmon of the Owenduff (Ballycroy) River." Proc. Roy. Irish Acad., **47**, Section B, No. 6, 1941.

SCIENTIFIC PROCEEDINGS.

VOLUME 22.

1.—Dissemination of Potato Viruses X and F by Leaf Contact. By J. B. LOUGHNANE and PAUL A. MURPHY, Albert Agricultural College, Glasnevin, Dublin. 1s. 6d. (May, 1938.)

2.—A Study of the Infiltration of Viruses into Seed Potato Stocks in the Field. By PHYLLIS CLINCH, J. B. LOUGHNANE, and PAUL A. MURPHY. 1s. 6d. (June, 1938.)

3.—Report of the Irish Radium Committee for the Year 1937. [Including Reports from The City of Dublin Skin and Cancer Hospital (*Radiologist*—DR. F. O. PILKINGTON); St. Anne's Hospital, Dublin (*Radiologist*—DR. OLIVER CHANCE); and by OLIVER CHANCE, M.B., B.CH., D.M.R.E., Richmond, Sir Patrick Dun's, Dr. Steevens', Rotunda, and Coombe Hospitals; and OSWALD J. MURPHY, M.B., B.CH., St. Vincent's Hospital, Dublin.] 1s. (July, 1938.)

4.—On a Recent Bog-flow in the County Wicklow. By G. F. MITCHELL. (Plate 1.) 1s. (July, 1938.)

5.—Subaqueous Transpiration. By H. H. DIXON, SC.D., F.R.S., Professor of Botany in the University of Dublin. 6d. (July, 1938.)

[Nos. 1 to 5, price 5s.]

6.—The Constitution of Laminarin. Isolation of 2 : 4 : 6-Trimethylglucopyranose. By VINCENT C. BARRY, M.SC., Chemical Laboratories, University College, Galway. 1s. (January, 1939.)

7.—A Study of the Seasonal Development of the Potato Plant in Relation to Blight Attack and Spraying. By PAUL A. MURPHY, Albert Agricultural College, Glasnevin, Dublin. 1s. (February, 1939.)

8.—Atmospheric Pollution in Dublin during the Year 1938. By A. G. G. LEONARD and BRIDGET P. MCVERRY, Chemical Department, University College, Dublin. 1s. (April, 1939.)

9.—The Ovule, Gametophytes, and Proembryo in *Saxegothaea*. By W. J. LOOBY and J. DOYLE (Botany Department, University College, Dublin). (Plates 2 and 3.) 3s. (May, 1939.)

10.—An Apparent Connection between the Presence of Colloids and the Osmotic Pressures of Conifer Leaves. By O. ROBERTS, M.SC., and S. A. STYLES, M.SC. 6d. (May, 1939.)

11.—Embryogeny in *Saxegothaea* and its Relation to other Podocarps. By J. DOYLE and W. J. LOOBY (Botany Department, University College, Dublin). 2s. (May, 1939.)

12.—"Studies in Peat." Part 5. Extraction of Peat with Azeotrope-like Petroleum mixed Solvents. By J. REILLY, D. F. KELLY, and J. DUFFY. 6d. (May, 1939.)

13.—Studies on the Ultimate Composition of Biological Material. Part III.—Glandular Tissues. By ROBERT PRESS and WILLIAM ROBERT FEARON. 6d. (May, 1939.)

14.—Report of the Irish Radium Committee for the Year 1938. Including Reports from OSWALD J. MURPHY, M.B., B.CH., St. Vincent's Hospital, Dublin; OLIVER CHANCE, M.B., B.CH., D.M.R.E., Richmond, Sir Patrick Dun's, Dr. Steevens', Rotunda, and Coombe Hospitals; and by St. Anne's Hospital, Dublin (*Radiologist*—DR. OLIVER CHANCE). 1s. (May, 1939.)

[Nos. 6 to 14, price, 10s.]

15.—Leaf Movement on Release of Sap Tension. By HENRY H. DIXON, SC.D., F.R.S., University Professor of Botany, Trinity College, Dublin, and J. S. BARLEE, B.SC., Assistant to the Professor of Botany, Trinity College, Dublin. (Plate 4.) 1s. 6d. (July, 1939.)

16.—"Studies in Peat." Part 6. The "Water Phase" in Azeotropic Extraction Solvents. By J. REILLY and D. F. KELLY. 6d. (July, 1939.)

17.—"Studies in Peat." Part 7. Water Distribution in Peat Wax Azeotropic Extraction Solvents. By J. REILLY, D. F. KELLY, J. DUFFY, and E. COUGHLAN. 6d. (July, 1939.)

[Nos. 15 to 17, price 2s.]

18.—On a Recent Bog-flow in Powerscourt Mountain Townland, Co. Wicklow. By A. D. DELAP and G. F. MITCHELL. 6d. (December, 1939.)

19.—The Chemical Constituents of Lichens found in Ireland. *Lecanora Gangaleoides*—Part 2. By T. J. NOLAN, B.A., D.SC., and J. KEANE, PH.D., University College, Dublin. 1s. (February, 1940.)

20.—Further Experiments on Transpiration into a Saturated Atmosphere. By HENRY H. DIXON, SC.D., F.R.S., Professor of Botany in Trinity College, Dublin, and J. S. BARLEE, B.SC., Lecturer in Biology, Royal Naval College, Dartmouth. (Plate 5.) 1s. 6d. (February, 1940.)

21.—Stabilised Diazo Complexes with Piperazine and other Bases. By P. J. DRUMM, W. F. O'CONNOR, and J. REILLY, Chemical Dept., University College, Cork. 6d. (March, 1940.)

22.—The Requirements of the Pig for Vitamins A and D. By BRENDAN J. SENIOR, M.SC., Animal Nutrition Department, University College, Dublin. (Plate 6.) 1s. (April, 1940.)

23.—The Chemical Constituents of the Lichen *Parmelia Latissima* Fée. By T. J. NOLAN, B.A., D.SC., J. KEANE, PH.D., and V. E. DAVIDSON, B.SC., University College, Dublin. 6d. (April, 1940.)

24.—New Observations on the Life-history of *Callitris*. By W. J. LOOBY and J. DOYLE, University College, Dublin. 1s. 6d. (May, 1940.)

[Nos. 18 to 24, price 6s.]

25.—Atmospheric Pollution in Dublin during the Year 1939. By A. G. G. LEONARD, BRIDGET P. MCVERRY, and D. CROWLEY. 1s. (May, 1940.)

26.—Studies in Peat. Part 8. Preliminary Note on Irish Peat Wax (Mona Wax). By J. REILLY and J. A. EMLYN. 6d. (May, 1940.)

27.—Report of the Irish Radium Committee for the Year 1939. Including Reports from OSWALD J. MURPHY, M.B., B.CH., St. Vincent's Hospital, Dublin; OLIVER CHANCE, M.B., B.CH., D.M.R.E., Richmond, Sir Patrick Dun's, Dr. Steevens', Rotunda, and Coombe Hospitals; DR. R. E. TOTTENHAM, City and County Hospital, Londonderry; St. Anne's Hospital, Dublin (*Radiologist*—DR. OLIVER CHANCE). 6d. (June, 1940.)

28.—The Production of Santonin from Irish-Grown Artemesia. By PETER J. DRUMM, PH.D., D.SC., and WILLIAM F. O'CONNOR, M.SC. 6d. (June, 1940.)

29.—A Molecular Constant for Soured Milks. II. Effect of Preservatives. By J. J. RYAN and G. T. PYNE (Department of Dairy Chemistry, University College, Cork). 6d. (June, 1940.)

30.—Award of the Boyle Medal to Professor Joseph Reilly.

31.—Salmon of the Ballisodare River. I.—History of the Ballisodare Fishery. By ARTHUR E. J. WENT, Department of Agriculture, Fisheries Branch, Dublin. (Plate No. 7.) 2s. 6d. (August, 1940.)

32.—Soils and Fresh-water Iodine-Content in Ireland in Relation to Endemic Goitre Incidence. By JAMES C. SHEE, Chemistry Department, University College, Cork. [Communicated by Professor J. Reilly.] 1s. (August, 1940.)

[Nos. 25 to 32, price 6s.]

33.—The Chlorination of Derivatives of o-Orsellinic Acid. By T. J. NOLAN, B.A., D.SC., and D. MURPHY, M.SC., University College, Dublin. 6d. (December, 1940.)

34.—"Studies in Peat." Part 9. The Cerotic and Carboceric Acid Fractions of Mona Wax. By PROFESSOR J. REILLY and JOHN P. WILSON, M.SC., Chemical Department, University College, Cork. 6d. (December, 1940.)

35.—Salmon of the Ballisodare River. II.—Age and Growth. By ARTHUR E. J. WENT, Department of Agriculture, Fisheries Branch, Dublin. 2s. 6d. (January, 1941.)

36.—The Evolution of the Atmosphere. By J. H. J. POOLE, SC.D. 2s. (February, 1941.)

37.—Studies in Peat. Part 10. Activated Charcoals from Peat Residue. By JOSEPH REILLY, D.SC., and JAMES R. O'DONOGHUE, M.SC., University College, Cork. 1s. (April, 1941.)

38.—The Vitamin D Requirements of the Growing Pig. By BRENDAN J. SENIOR, M.SC., Animal Nutrition Department, University College, Dublin. 6d. (April, 1941.)

[Nos. 33 to 38, price 6s.]

39.—Studies on the Oxidising Action of Selenious Acid. I. Organic Sulphur Compounds. By A. E. A. WERNER. 6d. (April, 1941.)

40.—The Effect of Wave-length on the Relation between the Intensity of Illumination and the Current in Selenium Rectifier Photo-Cells. By H. H. POOLE and W. R. G. ATKINS, F.R.S. 6d. (July, 1941.)

41.—Atmospheric Pollution in Dublin during the Year 1940. By A. G. G. LEONARD, BRIDGET P. MCVERRY, and D. CROWLEY. 6d. (July, 1941.)

42.—Estimation of Ammonia and Urea by a Modification of the Conway Diffusion Method. By EINHART KAWERAU, M.B., B.CH. From the Department of Physiology and Biochemistry, Trinity College, Dublin. 1s. (July, 1941.)

43.—The Phenol Amidine Reaction: The Detection of Guanidine, Guanidine Derivatives, and Urea, by Means of Thymol and Hypochlorite. By WILLIAM ROBERT FEARON. 6d. (July, 1941.)

44.—The Hydrolysis of Laminarin. Isolation of a New Glucose Disaccharide. By VINCENT C. BARRY, D.SC., University College, Galway. 6d. (July, 1941.)

45.—Report of the Irish Radium Committee for the Year 1940. Including Reports from OSWALD J. MURPHY, M.B., B.CH., St. Vincent's Hospital, Dublin; OLIVER CHANCE, M.B., B.CH., D.M.R.E., Richmond, Sir Patrick Dun's, Dr. Steevens', Rotunda, and Coombe Hospitals; R. E. TOTTENHAM, M.D., B.CH., B.A.O., City and County Hospital, Londonderry; St. Anne's Hospital, Dublin (*Radiologist*—DR. OLIVER CHANCE); and St. John's Hospital, Limerick (*Radiologist*—H. G. ROCHE KELLY, M.D., D.P.H.). (August, 1941.)

[Nos. 39 to 45, price 4s.]

46.—A Strain of the Tuber Blotch Virus causing Top Necrosis in Potato. By PHYLLIS E. M. CLINCH, Albert Agricultural College, Glasnevin. (Plate 8.) 1s. 6d. (December, 1941.)

47.—Phosphate Separation in Qualitative Analysis. By PROFESSOR J. REILLY and M. O'BRIEN, M.SC., Chemical Department, University College, Cork. 1s. (February, 1942.)

48.—Note on the Analysis of an Irish Phosphate Ore. By C. L. SIMPSON, M.SC., and J. REILLY, M.A., D.SC., F.I.C. 1s. (February, 1942.)

49.—Salmon of the River Erne. Results of the Examination of a small collection of Scales and Data. By ARTHUR E. J. WENT, Department of Agriculture, Fisheries Branch, Dublin. 1s. (March, 1942.)

[Nos. 46 to 49, price 4s.]

PRINTED BY FONSONBY AND GIBBS, UNIVERSITY PRESS, DUBLIN.